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(54) Title: INDUCING CELLULAR IMMUNE RESPONSES TO HEPATITIS C VIRUS USING PEPTIDE AND NUCLEIC ACID COMPOSITIONS

(57) Abstract: This invention uses our knowledge of the mechanisms by which antigen is recognized by T cells to identify and pre-
pare HCV epitopes, and to develop epitope-based vaccines directed towards HCV. More specifically, this application communicates
our discovery of pharmaceutical compositions and methods of use in the prevention and treatment of HCV infection.

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**INDUCING CELLULAR IMMUNE RESPONSES TO HEPATITIS C VIRUS
USING PEPTIDE AND NUCLEIC ACID COMPOSITIONS**

5

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- VII. Abstract

I. BACKGROUND OF THE INVENTION

Hepatitis C virus (HCV) infection is a global human health problem with approximately 150,000 new reported cases each year in the U.S. alone. HCV is a single stranded RNA virus, and is the etiological agent identified in most cases of non-A, non-B post-transfusion and post-transplant hepatitis, and is a common cause of acute sporadic hepatitis (Choo *et al.*, *Science* 244:359, 1989; Kuo *et al.*, *Science* 244:362, 1989; and Alter *et al.*, in: *Current Perspective in Hepatology*, p. 83, 1989). It is estimated that more than 50% of patients infected with HCV become chronically infected and, of those, 20% develop cirrhosis of the liver within 20 years (Davis *et al.*, *New Engl. J. Med.* 321:1501,

1989; Alter *et al.*, in: *Current Perspective in Hepatology*, p. 83, 1989; Alter *et al.*, *New Engl. J. Med.* 327:1899, 1992; and Dienstag, J. L. *Gastroenterology* 85:430, 1983).

Moreover, the only therapy available for treatment of HCV infection is interferon- α .

Most patients are unresponsive, however, and among the responders, there is a high

5 recurrence rate within 6-12 months of cessation of treatment (Liang *et al.*, *J. Med. Virol.* 40:69, 1993). Ribavirin, a guanosine analog with a broad spectrum activity against many RNA and DNA viruses, has been shown in clinical trials to be effective against chronic HCV infection when used in combination with interferon- α (*see, e.g.*, Poynard *et al.*, *Lancet* 352:1426-1432, 1998; Reichard *et al.*, *Lancet* 351:83-87, 1998) However, the
10 response rate is still well below 50%.

Virus-specific, human leukocyte antigen (HLA) class I-restricted cytotoxic T lymphocytes (CTL) are known to play a major role in the prevention and clearance of virus infections *in vivo* (Oldstone *et al.*, *Nature* 321:239, 1989; Jamieson *et al.*, *J. Virol.* 61:3930, 1987; Yap *et al.*, *Nature* 273:238, 1978; Lukacher *et al.*, *J. Exp. Med.* 160:814,
15 1994; McMichael *et al.*, *N. Engl. J. Med.* 309:13, 1983; Sethi *et al.*, *J. Gen. Virol.* 64:443, 1983; Watari *et al.*, *J. Exp. Med.* 165:459, 1987; Yasukawa *et al.*, *J. Immunol.* 143:2051, 1989; Tigges *et al.*, *J. Virol.* 66:1622, 1993; Reddenhase *et al.*, *J. Virol.* 55:263, 1985; Quinnan *et al.*, *N. Engl. J. Med.* 307:6, 1982). HLA class I molecules are expressed on the surface of almost all nucleated cells. Following intracellular processing of antigens,
20 epitopes from the antigens are presented as a complex with the HLA class I molecules on the surface of such cells. CTL recognize the peptide-HLA class I complex, which then results in the destruction of the cell bearing the HLA-peptide complex directly by the CTL and/or via the activation of non-destructive mechanisms *e.g.*, the production of interferon, that inhibit viral replication.

25 In view of the heterogeneous immune response observed with HCV infection, induction of a multi-specific cellular immune response directed simultaneously against multiple HCV epitopes appears to be important for the development of an efficacious vaccine against HCV. There is a need, however, to establish vaccine embodiments that elicit immune responses that correspond to responses seen in patients that clear HCV
30 infection.

The information provided in this section is intended to disclose the presently understood state of the art as of the filing date of the present application. Information is included in this section which was generated subsequent to the priority date of this

application. Accordingly, information in this section is not intended, in any way, to delineate the priority date for the invention.

II. SUMMARY OF THE INVENTION

5 This invention applies our knowledge of the mechanisms by which antigen is recognized by T cells, for example, to develop epitope-based vaccines directed towards HCV. More specifically, this application communicates our discovery of specific epitope pharmaceutical compositions and methods of use in the prevention and treatment of HCV infection.

10 Upon development of appropriate technology, the use of epitope-based vaccines has several advantages over current vaccines, particularly when compared to the use of whole antigens in vaccine compositions. There is evidence that the immune response to whole antigens is directed largely toward variable regions of the antigen, allowing for immune escape due to mutations. The epitopes for inclusion in an epitope-based vaccine
15 are selected from conserved regions of viral or tumor-associated antigens, which thereby reduces the likelihood of escape mutants. Furthermore, immunosuppressive epitopes that may be present in whole antigens can be avoided with the use of epitope-based vaccines.

An additional advantage of an epitope-based vaccine approach is the ability to combine selected epitopes (CTL and HTL), and further, to modify the composition of the
20 epitopes, achieving, for example, enhanced immunogenicity. Accordingly, the immune response can be modulated, as appropriate, for the target disease. Similar engineering of the response is not possible with traditional approaches.

Another major benefit of epitope-based immune-stimulating vaccines is their safety. The possible pathological side effects caused by infectious agents or whole
25 protein antigens, which might have their own intrinsic biological activity, is eliminated.

An epitope-based vaccine also provides the ability to direct and focus an immune response to multiple selected antigens from the same pathogen. Thus, patient-by-patient variability in the immune response to a particular pathogen may be alleviated by inclusion of epitopes from multiple antigens from that pathogen in a vaccine composition. A
30 "pathogen" may be an infectious agent or a tumor associated molecule.

One of the most formidable obstacles to the development of broadly efficacious epitope-based immunotherapeutics, however, has been the extreme polymorphism of HLA molecules. To date, effective non-genetically biased coverage of a population has been a task of considerable complexity; such coverage has required that epitopes be used

that are specific for HLA molecules corresponding to each individual HLA allele, therefore, impractically large numbers of epitopes would have to be used in order to cover ethnically diverse populations. Thus, there has existed a need for peptide epitopes that are bound by multiple HLA antigen molecules for use in epitope-based vaccines. The
5 greater the number of HLA antigen molecules bound, the greater the breadth of population coverage by the vaccine.

Furthermore, as described herein in greater detail, a need has existed to modulate peptide binding properties, for example, so that peptides that are able to bind to multiple HLA antigens do so with an affinity that will stimulate an immune response.

10 Identification of epitopes restricted by more than one HLA allele at an affinity that correlates with immunogenicity is important to provide thorough population coverage, and to allow the elicitation of responses of sufficient vigor to prevent or clear an infection in a diverse segment of the population. Such a response can also target a broad array of epitopes. The technology disclosed herein provides for such favored immune responses.

15 In a preferred embodiment, epitopes for inclusion in vaccine compositions of the invention are selected by a process whereby protein sequences of known antigens are evaluated for the presence of motif or supermotif-bearing epitopes. Peptides corresponding to a motif- or supermotif-bearing epitope are then synthesized and tested for the ability to bind to the HLA molecule that recognizes the selected motif. Those
20 peptides that bind at an intermediate or high affinity *i.e.*, an IC_{50} (or a K_D value) of 500 nM or less for HLA class I molecules or 1000 nM or less for HLA class II molecules, are further evaluated for their ability to induce a CTL or HTL response. Immunogenic peptide epitopes are selected for inclusion in vaccine compositions.

Supermotif-bearing peptides may additionally be tested for the ability to bind to
25 multiple alleles within the HLA supertype family. Moreover, peptide epitopes may be analogued to modify binding affinity and/or the ability to bind to multiple alleles within an HLA supertype.

The invention also includes an embodiment comprising a method for monitoring or evaluating an immune response to HCV in a patient having a known HLA-type, the
30 method comprising incubating a T lymphocyte sample from the patient with a peptide composition comprising an HCV epitope consisting essentially of an amino acid sequence described in Tables VII to Table XX or Table XXII which binds the product of at least one HLA allele present in said patient, and detecting for the presence of a T lymphocyte

that binds to the peptide. A CTL peptide epitope may, for example, comprise a tetrameric complex.

An alternative modality for defining the peptide epitopes in accordance with the invention is to recite the physical properties, such as length; primary structure; or charge, which are correlated with binding to a particular allele-specific HLA molecule or group of allele-specific HLA molecules. A further modality for defining peptide epitopes is to recite the physical properties of an HLA binding pocket, or properties shared by several allele-specific HLA binding pockets (*e.g.* pocket configuration and charge distribution) and reciting that the peptide epitope fits and binds to said pocket or pockets.

As will be apparent from the discussion below, other methods and embodiments are also contemplated. Further, novel synthetic peptides produced by any of the methods described herein are also part of the invention.

III. BRIEF DESCRIPTION OF THE FIGURES

Figure 1: Figure 1 provides a graph of total frequency of genotypes as a function of the number of HCV candidate epitopes bound by HLA-A and B molecules, in an average population.

Figure 2: Figure 2 illustrates the position of peptide epitopes in an experimental model minigene construct.

IV. DETAILED DESCRIPTION OF THE INVENTION

The peptide epitopes and corresponding nucleic acid compositions of the present invention are useful for stimulating an immune response to HCV by stimulating the production of CTL or HTL responses. The peptide epitopes, which are derived directly or indirectly from native HCV amino acid sequences, are able to bind to HLA molecules and stimulate an immune response to HCV. The complete polyprotein sequence from HCV and its variants can be obtained from Genbank. Peptide epitopes and analogs thereof can also be readily determined from sequence information that may subsequently be discovered for heretofore unknown variants of HCV, as will be clear from the disclosure provided below.

The peptide epitopes of the invention have been identified in a number of ways, as will be discussed below. Also discussed in greater detail is that analog peptides have been derived and the binding activity for HLA molecules modulated by modifying specific amino acid residues to create peptide analogs exhibiting altered immunogenicity.

Further, the present invention provides compositions and combinations of compositions that enable epitope-based vaccines that are capable of interacting with HLA molecules encoded by various genetic alleles to provide broader population coverage than prior vaccines.

5

IV.A. Definitions

The invention can be better understood with reference to the following definitions, which are listed alphabetically:

10 A "computer" or "computer system" generally includes: a processor; at least one information storage/retrieval apparatus such as, for example, a hard drive, a disk drive or a tape drive; at least one input apparatus such as, for example, a keyboard, a mouse, a touch screen, or a microphone; and display structure. Additionally, the computer may include a communication channel in communication with a network. Such a computer may include more or less than what is listed above.

15 "Cross-reactive binding" indicates that a peptide is bound by more than one HLA molecule; a synonym is degenerate binding.

A "cryptic epitope" elicits a response by immunization with an isolated peptide, but the response is not cross-reactive *in vitro* when intact whole protein which comprises the epitope is used as an antigen.

20 A "dominant epitope" is an epitope that induces an immune response upon immunization with a whole native antigen (see, *e.g.*, Sercarz, *et al.*, *Annu. Rev. Immunol.* 11:729-766, 1993). Such a response is cross-reactive *in vitro* with an isolated peptide epitope.

25 With regard to a particular amino acid sequence, an "epitope" is a set of amino acid residues which is involved in recognition by a particular immunoglobulin, or in the context of T cells, those residues necessary for recognition by T cell receptor proteins and/or Major Histocompatibility Complex (MHC) receptors. In an immune system setting, *in vivo* or *in vitro*, an epitope is the collective features of a molecule, such as primary, secondary and tertiary peptide structure, and charge, that together form a site
30 recognized by an immunoglobulin, T cell receptor or HLA molecule. Throughout this disclosure epitope and peptide are often used interchangeably.

It is to be appreciated that protein or peptide molecules that comprise an epitope of the invention as well as additional amino acid(s) are still within the bounds of the invention. In certain embodiments, there is a limitation on the length of a peptide of the

invention which is not otherwise a construct. An embodiment that is length-limited occurs when the protein/peptide comprising an epitope of the invention comprises a region (i.e., a contiguous series of amino acids) having 100% identity with a native sequence. In order to avoid the definition of epitope from reading, *e.g.*, on whole natural molecules, there is a limitation on the length of any region that has 100% identity with a native peptide sequence. Thus, for a peptide comprising an epitope of the invention and a region with 100% identity with a native peptide sequence (and is not otherwise a construct), the region with 100% identity to a native sequence generally has a length of: less than or equal to 600 amino acids, often less than or equal to 500 amino acids, often less than or equal to 400 amino acids, often less than or equal to 250 amino acids, often less than or equal to 100 amino acids, often less than or equal to 85 amino acids, often less than or equal to 75 amino acids, often less than or equal to 65 amino acids, and often less than or equal to 50 amino acids. In certain embodiments, an "epitope" of the invention is comprised by a peptide having a region with less than 51 amino acids that has 100% identity to a native peptide sequence, in any increment of (49, 48, 47, 46, 45, 44, 43, 42, 41, 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5) down to 5 amino acids.

Accordingly, peptide or protein sequences longer than 600 amino acids are within the scope of the invention, so long as they do not comprise any contiguous sequence of more than 600 amino acids that have 100% identity with a native peptide sequence, if they are not otherwise a construct. For any peptide that has five contiguous residues or less that correspond to a native sequence, there is no limitation on the maximal length of that peptide in order to fall within the scope of the invention. It is presently preferred that a CTL epitope be less than 600 residues long in any increment down to eight amino acid residues.

"Human Leukocyte Antigen" or "HLA" is a human class I or class II Major Histocompatibility Complex (MHC) protein (*see, e.g.*, Stites, *et al.*, IMMUNOLOGY, 8TH Ed., Lange Publishing, Los Altos, CA (1994).

An "HLA supertype or family", as used herein, describes sets of HLA molecules grouped on the basis of shared peptide-binding specificities. HLA class I molecules that share somewhat similar binding affinity for peptides bearing certain amino acid motifs are grouped into HLA supertypes. The terms HLA superfamily, HLA supertype family, HLA family, and HLA xx-like supertype molecules (where xx denotes a particular HLA type), are synonyms.

Throughout this disclosure, results are expressed in terms of "IC₅₀'s." IC₅₀ is the concentration of peptide in a binding assay at which 50% inhibition of binding of a reference peptide is observed. Given the conditions in which the assays are run (*i.e.*, limiting HLA proteins and labeled peptide concentrations), these values approximate K_D values. Assays for determining binding are described in detail, *e.g.*, in PCT publications WO 94/20127 and WO 94/03205. It should be noted that IC₅₀ values can change, often dramatically, if the assay conditions are varied, and depending on the particular reagents used (*e.g.*, HLA preparation, *etc.*). For example, excessive concentrations of HLA molecules will increase the apparent measured IC₅₀ of a given ligand.

Alternatively, binding is expressed relative to a reference peptide. Although as a particular assay becomes more, or less, sensitive, the IC₅₀'s of the peptides tested may change somewhat, the binding relative to the reference peptide will not significantly change. For example, in an assay run under conditions such that the IC₅₀ of the reference peptide increases 10-fold, the IC₅₀ values of the test peptides will also shift approximately 10-fold. Therefore, to avoid ambiguities, the assessment of whether a peptide is a good, intermediate, weak, or negative binder is generally based on its IC₅₀, relative to the IC₅₀ of a standard peptide.

Binding may also be determined using other assay systems including those using: live cells (*e.g.*, Ceppellini *et al.*, *Nature* 339:392, 1989; Christnick *et al.*, *Nature* 352:67, 1991; Busch *et al.*, *Int. Immunol.* 2:443, 1990; Hill *et al.*, *J. Immunol.* 147:189, 1991; del Guercio *et al.*, *J. Immunol.* 154:685, 1995), cell free systems using detergent lysates (*e.g.*, Cerundolo *et al.*, *J. Immunol.* 21:2069, 1991), immobilized purified MHC (*e.g.*, Hill *et al.*, *J. Immunol.* 152, 2890, 1994; Marshall *et al.*, *J. Immunol.* 152:4946, 1994), ELISA systems (*e.g.*, Reay *et al.*, *EMBO J.* 11:2829, 1992), surface plasmon resonance (*e.g.*, Khilko *et al.*, *J. Biol. Chem.* 268:15425, 1993); high flux soluble phase assays (Hammer *et al.*, *J. Exp. Med.* 180:2353, 1994), and measurement of class I MHC stabilization or assembly (*e.g.*, Ljunggren *et al.*, *Nature* 346:476, 1990; Schumacher *et al.*, *Cell* 62:563, 1990; Townsend *et al.*, *Cell* 62:285, 1990; Parker *et al.*, *J. Immunol.* 149:1896, 1992).

As used herein, "high affinity" with respect to HLA class I molecules is defined as binding with an IC₅₀, or K_D value, of 50 nM or less; "intermediate affinity" is binding with an IC₅₀ or K_D value of between about 50 and about 500 nM. "High affinity" with respect to binding to HLA class II molecules is defined as binding with an IC₅₀ or K_D value of 100 nM or less; "intermediate affinity" is binding with an IC₅₀ or K_D value of between about 100 and about 1000 nM.

The terms "identical" or percent "identity," in the context of two or more peptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues that are the same, when compared and aligned for maximum correspondence over a comparison window, as measured using a sequence comparison algorithm or by manual alignment and visual inspection.

An "immunogenic peptide" or "peptide epitope" is a peptide that comprises an allele-specific motif or supermotif such that the peptide will bind an HLA molecule and induce a CTL and/or HTL response. Thus, immunogenic peptides of the invention are capable of binding to an appropriate HLA molecule and thereafter inducing an HLA-restricted cytotoxic or helper T cell response to the antigen from which the immunogenic peptide is derived.

The phrases "isolated" or "biologically pure" refer to material which is substantially or essentially free from components which normally accompany the material as it is found in its native state. Thus, isolated peptides in accordance with the invention preferably do not contain materials normally associated with the peptides in their *in situ* environment. An "isolated" epitope refers to an epitope that does not include the whole sequence of the antigen or polypeptide from which the epitope was derived. Typically the "isolated" epitope does not have attached thereto additional amino acids that result in a sequence that has 100% identity with a native sequence. The native sequence can be a sequence such as a tumor-associated antigen from which the epitope is derived.

"Major Histocompatibility Complex" or "MHC" is a cluster of genes that plays a role in control of the cellular interactions responsible for physiologic immune responses. In humans, the MHC complex is also known as the HLA complex. For a detailed description of the MHC and HLA complexes, see, Paul, FUNDAMENTAL IMMUNOLOGY, 3RD ED., Raven Press, New York, 1993.

The term "motif" refers to the pattern of residues in a peptide of defined length, usually a peptide of from about 8 to about 13 amino acids for a class I HLA motif and from about 6 to about 25 amino acids for a class II HLA motif, which is recognized by a particular HLA molecule. Peptide motifs are typically different for each protein encoded by each human HLA allele and differ in the pattern of the primary and secondary anchor residues.

A "negative binding residue" is an amino acid which, if present at certain positions (typically not primary anchor positions) in a peptide epitope, results in decreased binding affinity of the peptide for the peptide's corresponding HLA molecule.

A "non-native" sequence or "construct" refers to a sequence that is not found in nature ("non-naturally occurring"). Such sequences include, *e.g.*, peptides that are lipidated or otherwise modified and polyepitopic compositions that contain epitopes that are non contiguous in a native protein sequence.

5 The term "peptide" is used interchangeably with "oligopeptide" in the present specification to designate a series of residues, typically L-amino acids, connected one to the other, typically by peptide bonds between the α -amino and carboxyl groups of adjacent amino acids. The preferred CTL-inducing peptides of the invention are 13 residues or less in length and usually consist of between about 8 and about 11 residues,
10 preferably 9 or 10 residues. The preferred HTL-inducing oligopeptides are less than about 50 residues in length and usually consist of between about 6 and about 30 residues, more usually between about 12 and 25, and often between about 15 and 20 residues.

"Pharmaceutically acceptable" refers to a generally non-toxic, inert, and/or physiologically compatible composition.

15 A "pharmaceutical excipient" comprises a material such as an adjuvant, a carrier, pH-adjusting and buffering agents, tonicity adjusting agents, wetting agents, preservative, and the like.

A "primary anchor residue" is an amino acid at a specific position along a peptide sequence which is understood to provide a contact point between the immunogenic
20 peptide and the HLA molecule. One to three, usually two, primary anchor residues within a peptide of defined length generally defines a "motif" for an immunogenic peptide. These residues are understood to fit in close contact with peptide binding grooves of an HLA molecule, with their side chains buried in specific pockets of the binding grooves themselves. In one embodiment, the primary anchor residues are located
25 at position 2 (from the amino terminal position) and at the carboxyl terminal position of a 9-residue peptide epitope in accordance with the invention. The primary anchor positions for each motif and supermotif are set forth in Table 1. For example, analog peptides can be created by altering the presence or absence of particular residues in these primary anchor positions. Such analogs are used to modulate the binding affinity of a peptide
30 comprising a particular motif or supermotif.

"Promiscuous recognition" is where a distinct peptide is recognized by the same T cell clone in the context of various HLA molecules. Promiscuous recognition or binding is synonymous with cross-reactive binding.

A "protective immune response" or "therapeutic immune response" refers to a CTL and/or an HTL response to an antigen derived from an infectious agent or a tumor antigen, which prevents or at least partially arrests disease symptoms or progression. The immune response may also include an antibody response which has been facilitated by the stimulation of helper T cells.

The term "residue" refers to an amino acid or amino acid mimetic incorporated into an oligopeptide by an amide bond or amide bond mimetic.

A "secondary anchor residue" is an amino acid at a position other than a primary anchor position in a peptide which may influence peptide binding. A secondary anchor residue occurs at a significantly higher frequency amongst bound peptides than would be expected by random distribution of amino acids at one position. The secondary anchor residues are said to occur at "secondary anchor positions." A secondary anchor residue can be identified as a residue which is present at a higher frequency among high or intermediate affinity binding peptides, or a residue otherwise associated with high or intermediate affinity binding. For example, analog peptides can be created by altering the presence or absence of particular residues in these secondary anchor positions. Such analogs are used to finely modulate the binding affinity of a peptide comprising a particular motif or supermotif.

A "subdominant epitope" is an epitope which evokes little or no response upon immunization with whole antigens which comprise the epitope, but for which a response can be obtained by immunization with an isolated peptide, and this response (unlike the case of cryptic epitopes) is detected when whole protein is used to recall the response *in vitro* or *in vivo*.

A "supermotif" is a peptide binding specificity shared by HLA molecules encoded by two or more HLA alleles. Preferably, a supermotif-bearing peptide is recognized with high or intermediate affinity (as defined herein) by two or more HLA antigens.

"Synthetic peptide" refers to a peptide that is man-made using such methods as chemical synthesis or recombinant DNA technology.

As used herein, a "vaccine" is a composition that contains one or more peptides of the invention. There are numerous embodiments of vaccines in accordance with the invention, such as by a cocktail of one or more peptides; one or more epitopes of the invention comprised by a polyepitopic peptide; or nucleic acids that encode such peptides or polypeptides, e.g., a minigene that encodes a polyepitopic peptide. The "one or more peptides" can include, e.g., at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18,

19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 100 or more peptides of the invention. The peptides or polypeptides can optionally be modified, such as by lipidation, addition of targeting or other sequences. HLA class I-binding peptides of the invention can be admixed with, or
5 linked to, HLA class II-binding peptides, to facilitate activation of both cytotoxic T lymphocytes and helper T lymphocytes. Vaccines can also comprise peptide-pulsed antigen presenting cells, e.g., dendritic cells.

The nomenclature used to describe peptide compounds follows the conventional practice wherein the amino group is presented to the left (the N-terminus) and the
10 carboxyl group to the right (the C-terminus) of each amino acid residue. When amino acid residue positions are referred to in a peptide epitope they are numbered in an amino to carboxyl direction with position one being the position closest to the amino terminal end of the epitope, or the peptide or protein of which it may be a part. In the formulae representing selected specific embodiments of the present invention, the amino- and
15 carboxyl-terminal groups, although not specifically shown, are in the form they would assume at physiologic pH values, unless otherwise specified. In the amino acid structure formulae, each residue is generally represented by standard three letter or single letter designations. The L-form of an amino acid residue is represented by a capital single letter or a capital first letter of a three-letter symbol, and the D-form for those amino acids
20 having D-forms is represented by a lower case single letter or a lower case three letter symbol. Glycine has no asymmetric carbon atom and is simply referred to as "Gly" or G. Symbols for the amino acids are shown below.

Single Letter Symbol	Three Letter Symbol	Amino Acids
A	Ala	Alanine
C	Cys	Cysteine
D	Asp	Aspartic Acid
E	Glu	Glutamic Acid
F	Phe	Phenylalanine
G	Gly	Glycine
H	His	Histidine
I	Ile	Isoleucine
K	Lys	Lysine
L	Leu	Leucine
M	Met	Methionine
N	Asn	Asparagine
P	Pro	Proline
Q	Gln	Glutamine
R	Arg	Arginine
S	Ser	Serine
T	Thr	Threonine
V	Val	Valine
W	Trp	Tryptophan
Y	Tyr	Tyrosine

IV.B. Stimulation of CTL and HTL responses

The mechanism by which T cells recognize antigens has been delineated during the past ten years. Based on our understanding of the immune system we have developed efficacious peptide epitope vaccine compositions that can induce a therapeutic or prophylactic immune response to HCV in a broad population. For an understanding of the value and efficacy of the claimed compositions, a brief review of immunology-related technology is provided.

A complex of an HLA molecule and a peptidic antigen acts as the ligand recognized by HLA-restricted T cells (Buus, S. *et al.*, *Cell* 47:1071, 1986; Babbitt, B. P. *et al.*, *Nature* 317:359, 1985; Townsend, A. and Bodmer, H., *Annu. Rev. Immunol.* 7:601,

1989; Germain, R. N., *Annu. Rev. Immunol.* 11:403, 1993). Through the study of single amino acid substituted antigen analogs and the sequencing of endogenously bound, naturally processed peptides, critical residues that correspond to motifs required for specific binding to HLA antigen molecules have been identified and are described herein and are set forth in Tables I, II, and III (*see also, e.g.,* Southwood, *et al., J. Immunol.* 160:3363, 1998; Rammensee, *et al., Immunogenetics* 41:178, 1995; Rammensee *et al., SYFPEITHI*, access via web at : <http://134.2.96.221/scripts.hlaserver.dll/home.htm>; Sette, A. and Sidney, J. *Curr. Opin. Immunol.* 10:478, 1998; Engelhard, V. H., *Curr. Opin. Immunol.* 6:13, 1994; Sette, A. and Grey, H. M., *Curr. Opin. Immunol.* 4:79, 1992; Sinigaglia, F. and Hammer, J. *Curr. Biol.* 6:52, 1994; Ruppert *et al., Cell* 74:929-937, 1993; Kondo *et al., J. Immunol.* 155:4307-4312, 1995; Sidney *et al., J. Immunol.* 157:3480-3490, 1996; Sidney *et al., Human Immunol.* 45:79-93, 1996; Sette, A. and Sidney, J. *Immunogenetics*, in press, 1999).

Furthermore, x-ray crystallographic analysis of HLA-peptide complexes has revealed pockets within the peptide binding cleft of HLA molecules which accommodate, in an allele-specific mode, residues borne by peptide ligands; these residues in turn determine the HLA binding capacity of the peptides in which they are present. (*See, e.g.,* Madden, D.R. *Annu. Rev. Immunol.* 13:587, 1995; Smith, *et al., Immunity* 4:203, 1996; Fremont *et al., Immunity* 8:305, 1998; Stern *et al., Structure* 2:245, 1994; Jones, E.Y. *Curr. Opin. Immunol.* 9:75, 1997; Brown, J. H. *et al., Nature* 364:33, 1993; Guo, H. C. *et al., Proc. Natl. Acad. Sci. USA* 90:8053, 1993; Guo, H. C. *et al., Nature* 360:364, 1992; Silver, M. L. *et al., Nature* 360:367, 1992; Matsumura, M. *et al., Science* 257:927, 1992; Madden *et al., Cell* 70:1035, 1992; Fremont, D. H. *et al., Science* 257:919, 1992; Saper, M. A., Bjorkman, P. J. and Wiley, D. C., *J. Mol. Biol.* 219:277, 1991.)

Accordingly, the definition of class I and class II allele-specific HLA binding motifs, or class I or class II supermotifs allows identification of regions within a protein that have the potential of binding particular HLA antigen(s).

The present inventors have found that the correlation of binding affinity with immunogenicity, which is disclosed herein, is an important factor to be considered when evaluating candidate peptides. Thus, by a combination of motif searches and HLA-peptide binding assays, candidates for epitope-based vaccines have been identified. After determining their binding affinity, additional confirmatory work can be performed to select, amongst these vaccine candidates, epitopes with preferred characteristics in terms of population coverage, antigenicity, and immunogenicity.

Various strategies can be utilized to evaluate immunogenicity, including:

- 1) Evaluation of primary T cell cultures from normal individuals (*see, e.g.,* Wentworth, P. A. *et al.*, *Mol. Immunol.* 32:603, 1995; Celis, E. *et al.*, *Proc. Natl. Acad. Sci. USA* 91:2105, 1994; Tsai, V. *et al.*, *J. Immunol.* 158:1796, 1997; Kawashima, I. *et al.*, *Human Immunol.* 59:1, 1998); This procedure involves the stimulation of peripheral blood lymphocytes (PBL) from normal subjects with a test peptide in the presence of antigen presenting cells *in vitro* over a period of several weeks. T cells specific for the peptide become activated during this time and are detected using, *e.g.*, a ^{51}Cr -release assay involving peptide sensitized target cells.
- 2) Immunization of HLA transgenic mice (*see, e.g.,* Wentworth, P. A. *et al.*, *J. Immunol.* 26:97, 1996; Wentworth, P. A. *et al.*, *Int. Immunol.* 8:651, 1996; Alexander, J. *et al.*, *J. Immunol.* 159:4753, 1997); In this method, peptides in incomplete Freund's adjuvant are administered subcutaneously to HLA transgenic mice. Several weeks following immunization, splenocytes are removed and cultured *in vitro* in the presence of test peptide for approximately one week. Peptide-specific T cells are detected using, *e.g.*, a ^{51}Cr -release assay involving peptide sensitized target cells and target cells expressing endogenously generated antigen.
- 3) Demonstration of recall T cell responses from immune individuals who have effectively been vaccinated, recovered from infection, and/or from chronically infected patients (*see, e.g.,* Rehermann, B. *et al.*, *J. Exp. Med.* 181:1047, 1995; Doolan, D. L. *et al.*, *Immunity* 7:97, 1997; Bertoni, R. *et al.*, *J. Clin. Invest.* 100:503, 1997; Threlkeld, S. C. *et al.*, *J. Immunol.* 159:1648, 1997; Diepolder, H. M. *et al.*, *J. Virol.* 71:6011, 1997). In applying this strategy, recall responses are detected by culturing PBL from subjects that have been naturally exposed to the antigen, for instance through infection, and thus have generated an immune response "naturally", or from patients who were vaccinated against the infection. PBL from subjects are cultured *in vitro* for 1-2 weeks in the presence of test peptide plus antigen presenting cells (APC) to allow activation of "memory" T cells, as compared to "naive" T cells. At the end of the culture period, T cell activity is detected using assays for T cell activity including ^{51}Cr release involving peptide-sensitized targets, T cell proliferation, or lymphokine release.

The following describes the peptide epitopes and corresponding nucleic acids of the invention.

IV.C. Binding Affinity of Peptide Epitopes for HLA Molecules

The large degree of HLA polymorphism is an important factor to consider with the epitope-based approach to vaccine development. To address this factor, epitope selection including identification of peptides capable of binding at high or intermediate
5 affinity to multiple HLA molecules is often utilized, most preferably these epitopes bind at high or intermediate affinity to two or more allele specific HLA molecules.

CTL-inducing peptides of interest for vaccine compositions preferably include those that have an IC_{50} or binding affinity value for class I HLA molecules of 500 nM or better (*i.e.*, the value is ≤ 500 nM). HTL-inducing peptides preferably include those that
10 have an IC_{50} or binding affinity value for class II HLA molecules of 1000 nM or better, (*i.e.*, the value is $\leq 1,000$ nM). For example, peptide binding is assessed by testing the capacity of a candidate peptide to bind to a purified HLA molecule *in vitro*. Peptides exhibiting high or intermediate affinity are then considered for further analysis. Selected peptides are tested on other members of the supertype family. In preferred embodiments,
15 peptides that exhibit cross-reactive binding are then used in vaccines or in cellular screening analyses.

Higher HLA binding affinity is typically correlated with greater immunogenicity. Greater immunogenicity can be manifested in several different ways. Immunogenicity corresponds to whether an immune response is elicited at all, and to the vigor of any
20 particular response, as well as to the extent of a population in which a response is elicited. For example, a peptide might elicit an immune response in a diverse array of the population, yet in no instance produce a vigorous response. In accordance with these principles, close to 90% of high binding peptides have been found to be immunogenic, as contrasted with about 50% of the peptides which bind with intermediate affinity.
25 Moreover, higher binding affinity peptides leads to more vigorous immunogenic responses. As a result, less peptide is required to elicit a similar biological effect if a high affinity binding peptide is used. Thus, in preferred embodiments of the invention, high affinity binding epitopes are particularly useful.

The relationship between binding affinity for HLA class I molecules and
30 immunogenicity of discrete peptide epitopes on bound antigens has been determined for the first time in the art by the present inventors. The correlation between binding affinity and immunogenicity was analyzed in two different experimental approaches (*see, e.g.*, Sette, *et al.*, *J. Immunol.* 153:5586-5592, 1994). In the first approach, the

immunogenicity of potential epitopes ranging in HLA binding affinity over a 10,000-fold range was analyzed in HLA-A*0201 transgenic mice. In the second approach, the antigenicity of approximately 100 different hepatitis B virus (HBV)-derived potential epitopes, all carrying A*0201 binding motifs, was assessed by using PBL from acute hepatitis patients. Pursuant to these approaches, it was determined that an affinity threshold value of approximately 500 nM (preferably 50 nM or less) determines the capacity of a peptide epitope to elicit a CTL response. These data are true for class I binding affinity measurements for naturally processed peptides and for synthesized T cell epitopes. These data also indicate the important role of determinant selection in the shaping of T cell responses (*see, e.g., Schaeffer et al. Proc. Natl. Acad. Sci. USA* 86:4649-4653, 1989).

An affinity threshold associated with immunogenicity in the context of HLA class II DR molecules has also been delineated (*see, e.g., Southwood et al. J. Immunology* 160:3363-3373, 1998). In order to define a biologically significant threshold of DR binding affinity, a database of the binding affinities of 32 DR-restricted epitopes for their restricting element (*i.e., the HLA molecule that binds the motif*) was compiled. In approximately half of the cases (15 of 32 epitopes), DR restriction was associated with high binding affinities, *i.e.* binding affinity values of 100 nM or less. In the other half of the cases (16 of 32), DR restriction was associated with intermediate affinity (binding affinity values in the 100-1000 nM range). In only one of 32 cases was DR restriction associated with an IC₅₀ of 1000 nM or greater. Thus, 1000 nM can be defined as an affinity threshold associated with immunogenicity in the context of DR molecules.

The binding affinity of peptides for HLA molecules can be determined as described in Example 1, below.

IV.D. Peptide Epitope Binding Motifs and Supermotifs

In the past few years evidence has accumulated to demonstrate that a large fraction of HLA class I and class II molecules can be classified into a relatively few supertypes, each characterized by largely overlapping peptide binding repertoires, and consensus structures of the main peptide binding pockets.

For HLA molecule pocket analyses, the residues comprising the B and F pockets of HLA class I molecules as described in crystallographic studies were analyzed (*see, e.g., Guo, H. C. et al., Nature* 360:364, 1992; Saper, M. A., Bjorkman, P. J. and Wiley, D. C., *J. Mol. Biol.* 219:277, 1991; Madden, D. R., Garboczi, D. N. and Wiley, D. C.,

Cell 75:693, 1993; Parham, P., Adams, E. J., and Arnett, K. L., *Immunol. Rev.* 143:141, 1995). In these analyses, residues 9, 45, 63, 66, 67, 70, and 99 were considered to make up the B pocket; and the B pocket was deemed to determine the specificity for the amino acid residue in the second position of peptide ligands. Similarly, residues 77, 80, 81, and 116 were considered to determine the specificity of the F pocket; the F pocket was deemed to determine the specificity for the C-terminal residue of a peptide ligand bound by the HLA class I molecule.

Through the study of single amino acid substituted antigen analogs and the sequencing of endogenously bound, naturally processed peptides, critical residues required for allele-specific binding to HLA molecules have been identified. The presence of these residues correlates with binding affinity for HLA molecules. The identification of motifs and/or supermotifs that correlate with high and intermediate affinity binding is an important issue with respect to the identification of immunogenic peptide epitopes for the inclusion in a vaccine. Kast *et al.* (*J. Immunol.* 152:3904-3912, 1994) have shown that motif-bearing peptides account for 90% of the epitopes that bind to allele-specific HLA class I molecules. In this study all possible peptides of 9 amino acids in length and overlapping by eight amino acids (240 peptides), which cover the entire sequence of the E6 and E7 proteins of human papillomavirus type 16, were evaluated for binding to five allele-specific HLA molecules that are expressed at high frequency among different ethnic groups. This unbiased set of peptides allowed an evaluation of the predictive value of HLA class I motifs. From the set of 240 peptides, 22 peptides were identified that bound to an allele-specific HLA molecule with high or intermediate affinity. Of these 22 peptides, 20 (*i.e.* 91%) were motif-bearing. Thus, this study demonstrates the value of motifs for the identification of peptide epitopes for inclusion in a vaccine: application of motif-based identification techniques eliminates screening of 90% of the potential epitopes in a target antigen protein sequence.

Such peptide epitopes are identified in the Tables described below.

Peptides of the present invention may also comprise epitopes that bind to MHC class II DR molecules. A greater degree of heterogeneity in both size and binding frame position of the motif, relative to the N and C termini of the peptide, exists for class II peptide ligands. This increased heterogeneity of HLA class II peptide ligands is due to the structure of the binding groove of the HLA class II molecule which, unlike its class I counterpart, is open at both ends. Crystallographic analysis of HLA class II DRB*0101-peptide complexes showed that the major energy of binding is contributed by peptide

residues complexed with complementary pockets on the DRB*0101 molecules. An important anchor residue engages the deepest hydrophobic pocket (*see, e.g.*, Madden, D.R. *Ann. Rev. Immunol.* 13:587, 1995) and is referred to as position 1 (P1). P1 may represent the N-terminal residue of a class II binding peptide epitope, but more typically is flanked towards the N-terminus by one or more residues. Other studies have also pointed to an important role for the peptide residue in the 6th position towards the C-terminus, relative to P1, for binding to various DR molecules.

Thus, peptides of the present invention are identified by any one of several HLA-specific amino acid motifs (*see, e.g.*, Tables I-III). If the presence of the motif corresponds to the ability to bind several allele-specific HLA antigens, it is referred to as a supermotif. The HLA molecules that bind to peptides that possess a particular amino acid supermotif are collectively referred to as an HLA "supertype."

The peptide motifs and supermotifs described below, and summarized in Tables I-III, provide guidance for the identification and use of peptide epitopes in accordance with the invention.

Examples of peptide epitopes bearing a respective supermotif or motif are included in Tables as designated in the description of each motif or supermotif below. The Tables include a binding affinity ratio listing for some of the peptide epitopes. The ratio may be converted to IC₅₀ by using the following formula: IC₅₀ of the standard peptide/ratio = IC₅₀ of the test peptide (*i.e.*, the peptide epitope). The IC₅₀ values of standard peptides used to determine binding affinities for Class I peptides are shown in Table IV. The IC₅₀ values of standard peptides used to determine binding affinities for Class II peptides are shown in Table V. The peptides used as standards for the binding assays described herein are examples of standards; alternative standard peptides can also be used when performing such an analysis.

To obtain the peptide epitope sequences listed in each Table, protein sequence data from fourteen HCV isolates were evaluated for the presence of the designated supermotif or motif. The fourteen strains include HPCCGAA, HPCPLYPRE, HCV-H-CMR, HCV-J1, HPCGENANTI, HPCGENOM, HPCHUMR, HPCJCG, HPCJTA, HCV-J483, HCV-JK1, HCV-N, HPCPOLP, and HCV-J8. Peptide epitopes were additionally evaluated on the basis of their conservancy among these fourteen strains. A criterion for conservancy requires that the entire sequence of an HLA class I binding peptide be totally conserved in 79% of the sequences available for a specific protein. Similarly, a criterion for conservancy requires that the entire 9-mer core region of an HLA class II binding

peptide be totally conserved in 79% of the sequences available for a specific protein. The percent conservancy of the selected peptide epitopes is indicated on the Tables. The frequency, *i.e.* the number of strains of the fourteen strains in which the totally conserved peptide sequence was identified, is also shown. The "position" column in the Tables
 5 designates the amino acid position of the HCV polyprotein that corresponds to the first amino acid residue of the epitope. The "number of amino acids" indicates the number of residues in the epitope sequence.

HLA Class I Motifs Indicative of CTL Inducing Peptide Epitopes:

10 The primary anchor residues of the HLA class I peptide epitope supermotifs and motifs delineated below are summarized in Table I. The HLA class I motifs set out in Table I(a) are those most particularly relevant to the invention claimed here. Primary and secondary anchor positions are summarized in Table II. Allele-specific HLA molecules that comprise HLA class I supertype families are listed in Table VI.

15

IV.D.1. HLA-A1 supermotif

The HLA-A1 supermotif is characterized by the presence in peptide ligands of a small (T or S) or hydrophobic (L, I, V, or M) primary anchor residue in position 2, and an aromatic (Y, F, or W) primary anchor residue at the C-terminal position of the epitope.
 20 The corresponding family of HLA molecules that bind to the A1 supermotif (*i.e.*, the HLA-A1 supertype) includes at least A*0101, A*2601, A*2602, A*2501, and A*3201 (*see, e.g.*, DiBrino, M. *et al.*, *J. Immunol.* 151:5930, 1993; DiBrino, M. *et al.*, *J. Immunol.* 152:620, 1994; Kondo, A. *et al.*, *Immunogenetics* 45:249, 1997). Other allele-specific HLA molecules predicted to be members of the A1 superfamily are shown in
 25 Table VI. Peptides binding to each of the individual HLA proteins can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

Peptide epitopes that comprise the A1 supermotif are set forth in Table VII.

30 IV.D.2. HLA-A2 supermotif

Primary anchor specificities for allele-specific HLA-A2.1 molecules (Falk *et al.*, *Nature* 351:290-296, 1991; Hunt *et al.*, *Science* 255:1261-1263, 1992; Parker *et al.*, *J. Immunol.* 149:3580-3587, 1992) and cross-reactive binding within the HLA A2 family (Fruci *et al.*, *Human Immunol.* 38:187-192, 1993; Tanigaki *et al.*, *Human Immunol.*

39:155-162, 1994) have been described. The present inventors have defined additional primary anchor residues that determine cross-reactive binding to multiple allele-specific HLA A2 molecules (Ruppert *et al.*, *Cell* 74:929-937, 1993; Del Guercio *et al.*, *J. Immunol.* 154:685-693, 1995; Kast *et al.*, *J. Immunol.* 152:3904-3912, 1994). The HLA-
5 A2 supermotif comprises peptide ligands with L, I, V, M, A, T, or Q as a primary anchor residue at position 2 and L, I, V, M, A, or T as a primary anchor residue at the C-terminal position of the epitope.

The corresponding family of HLA molecules (*i.e.*, the HLA-A2 supertype that binds these peptides) is comprised of at least: A*0201, A*0202, A*0203, A*0204,
10 A*0205, A*0206, A*0207, A*0209, A*0214, A*6802, and A*6901. Other allele-specific HLA molecules predicted to be members of the A2 superfamily are shown in Table VI. As explained in detail below, binding to each of the individual allele-specific HLA molecules can be modulated by substitutions at the primary anchor and/or secondary anchor positions, preferably choosing respective residues specified for the
15 supermotif.

Peptide epitopes that comprise an A2 supermotif are set forth in Table VIII. The motifs comprising the primary anchor residues V, A, T, or Q at position 2 and L, I, V, A, or T at the C-terminal position are those most particularly relevant to the invention claimed herein.
20

IV.D.3. HLA-A3 supermotif

The HLA-A3 supermotif is characterized by the presence in peptide ligands of A, L, I, V, M, S, or, T as a primary anchor at position 2, and a positively charged residue, R or K, at the C-terminal position of the epitope (*e.g.*, in position 9 of 9-mers). Exemplary
25 members of the corresponding family of HLA molecules (the HLA-A3 supertype) that bind the A3 supermotif include at least A*0301, A*1101, A*3101, A*3301, and A*6801. Other allele-specific HLA molecules predicted to be members of the A3 superfamily are shown in Table VI. As explained in detail below, peptide binding to each of the individual allele-specific HLA proteins can be modulated by substitutions of amino acids
30 at the primary and/or secondary anchor positions of the peptide, preferably choosing respective residues specified for the supermotif.

Peptide epitopes that comprise the A3 supermotif are set forth in Table IX.

IV.D.4. HLA-A24 supermotif

The HLA-A24 supermotif is characterized by the presence in peptide ligands of an aromatic (F, W, or Y) or hydrophobic aliphatic (L, I, V, M, or T) residue as a primary anchor in position 2, and Y, F, W, L, I, or M as primary anchor at the C-terminal position of the epitope. The corresponding family of HLA molecules that bind to the A24 supermotif (*i.e.*, the A24 supertype) includes at least A*2402, A*3001, and A*2301. Other allele-specific HLA molecules predicted to be members of the A24 superfamily are shown in Table VI. Peptide binding to each of the allele-specific HLA molecules can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

Peptide epitopes that comprise the A24 supermotif are set forth in Table X.

IV.D.5. HLA-B7 supermotif

The HLA-B7 supermotif is characterized by peptides bearing proline in position 2 as a primary anchor, and a hydrophobic or aliphatic amino acid (L, I, V, M, A, F, W, or Y) as the primary anchor at the C-terminal position of the epitope. The corresponding family of HLA molecules that bind the B7 supermotif (*i.e.*, the HLA-B7 supertype) is comprised of at least twenty six HLA-B proteins including: B*0702, B*0703, B*0704, B*0705, B*1508, B*3501, B*3502, B*3503, B*3504, B*3505, B*3506, B*3507, B*3508, B*5101, B*5102, B*5103, B*5104, B*5105, B*5301, B*5401, B*5501, B*5502, B*5601, B*5602, B*6701, and B*7801 (*see, e.g.*, Sidney, *et al.*, *J. Immunol.* 154:247, 1995; Barber, *et al.*, *Curr. Biol.* 5:179, 1995; Hill, *et al.*, *Nature* 360:434, 1992; Rammensee, *et al.*, *Immunogenetics* 41:178, 1995). Other allele-specific HLA molecules predicted to be members of the B7 superfamily are shown in Table VI. As explained in detail below, peptide binding to each of the individual allele-specific HLA proteins can be modulated by substitutions at the primary and/or secondary anchor positions of the peptide, preferably choosing respective residues specified for the supermotif.

Peptide epitopes that comprise the B7 supermotif are set forth in Table XI.

IV.D.6. HLA-B27 supermotif

The HLA-B27 supermotif is characterized by the presence in peptide ligands of a positively charged (R, H, or K) residue as a primary anchor at position 2, and a hydrophobic (F, Y, L, W, M, I, A, or V) residue as a primary anchor at the C-terminal position of the epitope. Exemplary members of the corresponding family of HLA

molecules that bind to the B27 supermotif (*i.e.*, the B27 supertype) include at least B*1401, B*1402, B*1509, B*2702, B*2703, B*2704, B*2705, B*2706, B*3801, B*3901, B*3902, and B*7301. Other allele-specific HLA molecules predicted to be members of the B27 superfamily are shown in Table VI. Peptide binding to each of the allele-specific HLA molecules can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

Peptide epitopes that comprise the B27 supermotif are set forth in Table XII.

10 IV.D.7. HLA-B44 supermotif

The HLA-B44 supermotif is characterized by the presence in peptide ligands of negatively charged (D or E) residues as a primary anchor in position 2, and hydrophobic residues (F, W, Y, L, I, M, V, or A) as a primary anchor at the C-terminal position of the epitope. Exemplary members of the corresponding family of HLA molecules that bind to the B44 supermotif (*i.e.*, the B44 supertype) include at least: B*1801, B*1802, B*3701, B*4001, B*4002, B*4006, B*4402, B*4403, and B*4006. Peptide binding to each of the allele-specific HLA molecules can be modulated by substitutions at primary and/or secondary anchor positions; preferably choosing respective residues specified for the supermotif.

20

IV.D.8. HLA-B58 supermotif

The HLA-B58 supermotif is characterized by the presence in peptide ligands of a small aliphatic residue (A, S, or T) as a primary anchor residue at position 2, and an aromatic or hydrophobic residue (F, W, Y, L, I, V, M, or A) as a primary anchor residue at the C-terminal position of the epitope. Exemplary members of the corresponding family of HLA molecules that bind to the B58 supermotif (*i.e.*, the B58 supertype) include at least: B*1516, B*1517, B*5701, B*5702, and B*5801. Other allele-specific HLA molecules predicted to be members of the B58 superfamily are shown in Table VI. Peptide binding to each of the allele-specific HLA molecules can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

30

Peptide epitopes that comprise the B58 supermotif are set forth in Table XIII.

IV.D.9. HLA-B62 supermotif

The HLA-B62 supermotif is characterized by the presence in peptide ligands of the polar aliphatic residue Q or a hydrophobic aliphatic residue (L, V, M, I, or P) as a primary anchor in position 2, and a hydrophobic residue (F, W, Y, M, I, V, L, or A) as a primary anchor at the C-terminal position of the epitope. Exemplary members of the corresponding family of HLA molecules that bind to the B62 supermotif (*i.e.*, the B62 supertype) include at least: B*1501, B*1502, B*1513, and B5201. Other allele-specific HLA molecules predicted to be members of the B62 superfamily are shown in Table VI. Peptide binding to each of the allele-specific HLA molecules can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

Peptide epitopes that comprise the B62 supermotif are set forth in Table XIV.

IV.D.10. HLA-A1 motif

The HLA-A1 motif is characterized by the presence in peptide ligands of T, S, or M as a primary anchor residue at position 2 and the presence of Y as a primary anchor residue at the C-terminal position of the epitope. An alternative allele-specific A1 motif is characterized by a primary anchor residue at position 3 rather than position 2. This motif is characterized by the presence of D, E, A, or S as a primary anchor residue in position 3, and a Y as a primary anchor residue at the C-terminal position of the epitope. Peptide binding to HLA A1 can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the motif.

Peptide epitopes that comprise either A1 motif are set forth in Table XV. The epitopes comprising T, S, or M at position 2 and Y at the C-terminal position are also included in the listing of HLA-A1 supermotif-bearing peptide epitopes listed in Table VII.

IV.D.11. HLA-A*0201 motif

An HLA-A2*0201 motif was first determined to be characterized by the presence in peptide ligands of L or M as a primary anchor residue in position 2, and L or V as a primary anchor residue at the C-terminal position of a 9-residue peptide (Falk *et al.*, *Nature* 351:290-296, 1991). The A*0201 motif was also determined to further comprise an I at position 2 and I or A at the C-terminal position of a nine amino acid peptide (Hunt

et al., Science 255:1261-1263, March 6, 1992; Parker *et al.*, *J. Immunol.* 149:3580-3587, 1992). Subsequently, the A*0201 allele-specific motif has been defined by the present inventors to additionally comprise V, A, T, or Q as a primary anchor residue at position 2, and M as a primary anchor residue at the C-terminal position of the epitope.

- 5 Additionally, the A*0201 allele-specific motif has been found to comprise a T at the C-terminal position (Kast *et al.*, *J. Immunol.* 152:3904-3912, 1994). Thus, the HLA-A*0201 motif comprises peptide ligands with L, I, V, M, A, T, or Q as primary anchor residues at position 2 and L, I, V, M, A, or T as a primary anchor residue at the C-terminal position of the epitope. The preferred and tolerated residues that characterize the
- 10 primary anchor positions of the HLA-A*0201 motif are identical to the residues describing the A2 supermotif. (For reviews of relevant data, *see, e.g.*, Del Guercio *et al.*, *J. Immunol.* 154:685-693, 1995; Ruppert *et al.*, *Cell* 74:929-937, 1993; Sidney *et al.*, *Immunol. Today* 17:261-266, 1996; Sette and Sidney, *Curr. Opin. in Immunol.* 10:478-482, 1998). Secondary anchor residues that characterize the A*0201 motif have
- 15 additionally been defined as disclosed herein. These are disclosed in Table II. Peptide binding to HLA-A*0201 molecules can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the motif.

- Peptide epitopes that comprise an A*0201 motif are set forth in Table VIII. The
- 20 A*0201 motifs comprising the primary anchor residues V, A, T, or Q at position 2 and L, I, V, A, or T at the C-terminal position are those most particularly relevant to the invention claimed herein.

IV.D.12. HLA-A3 motif

- 25 The HLA-A3 motif is characterized by the presence in peptide ligands of L, M, V, I, S, A, T, F, C, G, or D as a primary anchor residue at position 2, and the presence of K, Y, R, H, F, or A as a primary anchor residue at the C-terminal position of the epitope. Peptide binding to HLA-A3 can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the
- 30 motif.

The A3 supermotif primary anchor residues comprise a subset of the A3- and A11-allele specific motif primary anchor residues. Peptide epitopes that comprise the A3 motif are set forth in Table XVI. Those peptide epitopes that also comprise the A3 supermotif are also listed in Table IX.

IV.D.13. HLA-A11 motif

The HLA-A11 motif is characterized by the presence in peptide ligands of V, T, M, L, I, S, A, G, N, C, D, or F as a primary anchor residue in position 2, and K, R, Y, or H as a primary anchor residue at the C-terminal position of the epitope. Peptide binding to HLA-A11 can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the motif.

Peptide epitopes that comprise the A11 motif are set forth in Table XVII; peptide epitopes comprising the A3 allele-specific motif are also present in this Table because of the overlap between the A3 and A11 motif primary anchor specificities. Further, those peptide epitopes that comprise the A3 supermotif are also listed in Table IX.

IV.D.14. HLA-A24 motif

The HLA-A24 motif is characterized by the presence in peptide ligands of Y, F, W, or M as a primary anchor residue in position 2, and F, L, I, or W as a primary anchor residue at the C-terminal position of the epitope. Peptide binding to HLA-A24 molecules can be modulated by substitutions at primary and/or secondary anchor positions; preferably choosing respective residues specified for the motif.

Peptide epitopes that comprise the A24 motif are set forth in Table XVIII. These epitopes are also listed in Table X, which sets forth HLA-A24-supermotif-bearing peptide epitopes, as the primary anchor residues characterizing the A24 allele-specific motif comprise a subset of the A24 supermotif primary anchor residues.

HLA Class II Binding Motifs

The primary and secondary anchor residues of the HLA class II peptide epitope supermotifs and motifs delineated below are summarized in Table III.

IV.D.15. HLA DR-1-4-7 supermotif

Motifs have also been identified for peptides that bind to three common HLA class II allele-specific HLA molecules: HLA DRB1*0401, DRB1*0101, and DRB1*0701. Collectively, the common residues from these motifs delineate the HLA DR-1-4-7 supermotif. Peptides that bind to these DR molecules carry a supermotif characterized by a large aromatic or hydrophobic residue (Y, F, W, L, I, V, or M) as a primary anchor residue in position 1, and a small, non-charged residue (S, T, C, A, P, V,

I, L, or M) as a primary anchor residue in position 6 of a 9-mer core region. Allele-specific secondary effects and secondary anchors for each of these HLA types have also been identified. These are set forth in Table III. Peptide binding to HLA- DRB1*0401, DRB1*0101, and/or DRB1*0701 can be modulated by substitutions at primary and/or
5 secondary anchor positions, preferably choosing respective residues specified for the supermotif.

Conserved peptide epitopes *i.e.*, conserved in $\geq 79\%$ ($\geq 11/14$) of the HCV strains used for the present analysis, may be described as corresponding to epitopes containing a nine residue core comprising the DR-1-4-7 supermotif, and in which the 9 residue core is
10 conserved in $\geq 79\%$ (wherein position 1 of the motif is at position 1 of the nine residue core). Conserved 9-mer core regions are set forth in Table XIXa. Respective exemplary peptide epitopes of 15 amino acid residues in length, each of which comprise a conserved nine residue core, are also shown in section "a" of the Table. Cross-reactive binding data for exemplary 15-residue supermotif-bearing peptides are shown in Table XIXb.

15

IV.D.16. HLA DR3 motifs

Two alternative motifs (*i.e.*, submotifs) characterize peptide epitopes that bind to HLA-DR3 molecules. In the first motif (submotif DR3A) a large, hydrophobic residue (L, I, V, M, F, or Y) is present in anchor position 1 of a 9-mer core, and D is present as an
20 anchor at position 4, towards the carboxyl terminus of the epitope. As in other class II motifs, core position 1 may or may not occupy the peptide N-terminal position.

The alternative DR3 submotif provides for lack of the large, hydrophobic residue at anchor position 1, and/or lack of the negatively charged or amide-like anchor residue at position 4, by the presence of a positive charge at position 6 towards the carboxyl
25 terminus of the epitope. Thus, for the alternative allele-specific DR3 motif (submotif DR3B): L, I, V, M, F, Y, A, or Y is present at anchor position 1; D, N, Q, E, S, or T is present at anchor position 4; and K, R, or H is present at anchor position 6. Peptide binding to HLA-DR3 can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the motif.

30 Conserved 9-mer core regions (*i.e.*, those sequences that are conserved in at least 79% of the 14 HCV strains used for the analysis) corresponding to a nine residue sequence comprising the DR3A submotif (wherein position 1 of the motif is at position 1 of the nine residue core) are set forth in Table XXa. Respective exemplary peptide

epitopes of 15 amino acid residues in length, each of which comprise a conserved nine residue core, are also shown in Table XXa. Table XXb shows binding data of exemplary DR3 submotif A-bearing peptides.

Conserved 9-mer core regions (*i.e.*, those that are at least 79% conserved in the 14
5 HCV strains used for the analysis) comprising the DR3B submotif and respective
exemplary 15-mer peptides comprising the DR3 submotif-B epitope are set forth in Table
XXc. Table XXd shows binding data of exemplary DR3 submotif B-bearing peptides.

Each of the HLA class I or class II peptide epitopes set out in the Tables herein
are deemed singly to be an inventive aspect of this application. Further, it is also an
10 inventive aspect of this application that each peptide epitope may be used in combination
with any other peptide epitope.

IV.E. Enhancing Population Coverage of the Vaccine

Vaccines that have broad population coverage are preferred because they are more
15 commercially viable and generally applicable to the most people. Broad population
coverage can be obtained using the peptides of the invention (and nucleic acid
compositions that encode such peptides) through selecting peptide epitopes that bind to
HLA alleles which, when considered in total, are present in most of the population. Table
XXI lists the overall frequencies of the HLA class I supertypes in various ethnicities
20 (Table XXIa) and the combined population coverage achieved by the A2-, A3-, and B7-
supertypes (Table XXIb). The A2-, A3-, and B7 supertypes are each present on the
average of over 40% in each of these five major ethnic groups. Coverage in excess of
80% is achieved with a combination of these supermotifs. These results suggest that
effective and non-ethnically biased population coverage is achieved upon use of a limited
25 number of cross-reactive peptides. Although the population coverage reached with these
three main peptide specificities is high, coverage can be expanded to reach 95%
population coverage and above, and more easily achieve truly multispecific responses
upon use of additional supermotif or allele-specific motif bearing peptides.

The B44-, A1-, and A24-supertypes are present, on average, in a range from 25%
30 to 40% in these major ethnic populations (Table XXIa). While less prevalent overall, the
B27-, B58-, and B62 supertypes are each present with a frequency >25% in at least one
major ethnic group (Table XXIa). Table XXIb summarizes the estimated prevalence of
combinations of HLA supertypes that have been identified in five major ethnic groups.

The incremental coverage obtained by the inclusion of A1-, A24-, and B44-supertypes to the A2, A3, and B7 coverage, or all of the supertypes described herein, is shown.

The data presented herein, together with the previous definition of the A2-, A3-, and B7-supertypes, indicates that all antigens, with the possible exception of A29, B8, and B46, can be classified into a total of nine HLA supertypes. By including epitopes from the six most frequent supertypes, an average population coverage of 99% is obtained for five major ethnic groups..

IV.F. Immune Response-Stimulating Peptide Analogs

In general, CTL and HTL responses are not directed against all possible epitopes. Rather, they are restricted to a few "immunodominant" determinants (Zinkernagel, *et al.*, *Adv. Immunol.* 27:5159, 1979; Bennink, *et al.*, *J. Exp. Med.* 168:1935-1939, 1988; Rawle, *et al.*, *J. Immunol.* 146:3977-3984, 1991). It has been recognized that immunodominance (Benacerraf, *et al.*, *Science* 175:273-279, 1972) could be explained by either the ability of a given epitope to selectively bind a particular HLA protein (determinant selection theory) (Vitiello, *et al.*, *J. Immunol.* 131:1635, 1983); Rosenthal, *et al.*, *Nature* 267:156-158, 1977), or to be selectively recognized by the existing TCR (T cell receptor) specificities (repertoire theory) (Klein, J., *IMMUNOLOGY, THE SCIENCE OF SELF/NONSELF DISCRIMINATION*, John Wiley & Sons, New York, pp. 270-310, 1982). It has been demonstrated that additional factors, mostly linked to processing events, can also play a key role in dictating, beyond strict immunogenicity, which of the many potential determinants will be presented as immunodominant (Sercarz, *et al.*, *Annu. Rev. Immunol.* 11:729-766, 1993).

The concept of dominance and subdominance is relevant to immunotherapy of both infectious diseases and cancer. For example, in the course of chronic viral disease, recruitment of subdominant epitopes can be important for successful clearance of the infection, especially if dominant CTL or HTL specificities have been inactivated by functional tolerance, suppression, mutation of viruses and other mechanisms (Franco, *et al.*, *Curr. Opin. Immunol.* 7:524-531, 1995). In the case of cancer and tumor antigens, CTLs recognizing at least some of the highest binding affinity peptides might be functionally inactivated. Lower binding affinity peptides are preferentially recognized at these times, and may therefore be preferred in therapeutic or prophylactic anti-cancer vaccines.

In particular, it has been noted that a significant number of epitopes derived from known non-viral tumor associated antigens (TAA) bind HLA class I with intermediate affinity (IC_{50} in the 50-500 nM range). For example, it has been found that 8 of 15 known TAA peptides recognized by tumor infiltrating lymphocytes (TIL) or CTL bound in the 50-500 nM range. (These data are in contrast with estimates that 90% of known viral antigens were bound by HLA class I molecules with IC_{50} of 50 nM or less, while only approximately 10% bound in the 50-500 nM range (Sette, *et al.*, *J. Immunol.*, 153:558-5592, 1994). In the cancer setting this phenomenon is probably due to elimination or functional inhibition of the CTL recognizing several of the highest binding peptides, presumably because of T cell tolerization events.

Without intending to be bound by theory, it is believed that because T cells to dominant epitopes may have been clonally deleted, selecting subdominant epitopes may allow existing T cells to be recruited, which will then lead to a therapeutic or prophylactic response. However, the binding of HLA molecules to subdominant epitopes is often less vigorous than to dominant ones. Accordingly, there is a need to be able to modulate the binding affinity of particular immunogenic epitopes for one or more HLA molecules, and thereby to modulate the immune response elicited by the peptide, for example to prepare analog peptides which elicit a more vigorous response. This ability would greatly enhance the usefulness of peptide-based vaccines and therapeutic agents.

Although peptides with suitable cross-reactivity among all alleles of a superfamily are identified by the screening procedures described above, cross-reactivity is not always as complete as possible, and in certain cases procedures to increase cross-reactivity of peptides can be useful; moreover, such procedures can also be used to modify other properties of the peptides such as binding affinity or peptide stability. Having established the general rules that govern cross-reactivity of peptides for HLA alleles within a given motif or supermotif, modification (*i.e.*, analoging) of the structure of peptides of particular interest in order to achieve broader (or otherwise modified) HLA binding capacity can be performed. More specifically, peptides which exhibit the broadest cross-reactivity patterns, can be produced in accordance with the teachings herein. The present concepts related to analog generation are set forth in greater detail in co-pending U.S.S.N. 09/226,775 filed 1/6/99.

In brief, the strategy employed utilizes the motifs or supermotifs which correlate with binding to certain HLA molecules. The motifs or supermotifs are defined by having primary anchors, and in many cases secondary anchors. Analog peptides can be created

by substituting amino acid residues at primary anchor, secondary anchor, or at primary and secondary anchor positions. Generally, analogs are made for peptides that already bear a motif or supermotif. Preferred secondary anchor residues of supermotifs and motifs that have been defined for HLA class I and class II binding peptides are shown in
5 Tables II and III, respectively.

For a number of the motifs or supermotifs in accordance with the invention, residues are defined which are deleterious to binding to allele-specific HLA molecules or members of HLA supertypes that bind the respective motif or supermotif (Tables II and III). Accordingly, removal of such residues that are detrimental to binding can be
10 performed in accordance with the present invention. For example, in the case of the A3 supertype, when all peptides that have such deleterious residues are removed from the population of analyzed peptides, the incidence of cross-reactivity increases from 22% to 37% (see, e.g., Sidney, J. *et al.*, *Hu. Immunol.* 45:79, 1996). Thus, one strategy to improve the cross-reactivity of peptides within a given supermotif is simply to delete one
15 or more of the deleterious residues present within a peptide and substitute a small "neutral" residue such as Ala (that may not influence T cell recognition of the peptide). An enhanced likelihood of cross-reactivity is expected if, together with elimination of detrimental residues within a peptide, "preferred" residues associated with high affinity binding to an allele-specific HLA molecule or to multiple HLA molecules within a
20 superfamily are inserted.

To ensure that an analog peptide, when used as a vaccine, actually elicits a CTL response to the native epitope *in vivo* (or, in the case of class II epitopes, elicits helper T cells that cross-react with the wild type peptides), the analog peptide may be used to immunize T cells *in vitro* from individuals of the appropriate HLA allele. Thereafter, the
25 immunized cells' capacity to induce lysis of wild type peptide sensitized target cells is evaluated. It will be desirable to use as antigen presenting cells, cells that have been either infected, or transfected with the appropriate genes, or, in the case of class II epitopes only, cells that have been pulsed with whole protein antigens, to establish whether endogenously produced antigen is also recognized by the relevant T cells.

Another embodiment of the invention is to create analogs of weak binding peptides, to thereby ensure adequate numbers of cross-reactive cellular binders. Class I binding peptides exhibiting binding affinities of 500-5000 nM, and carrying an acceptable but suboptimal primary anchor residue at one or both positions can be "fixed" by
30

substituting preferred anchor residues in accordance with the respective supertype. The analog peptides can then be tested for crossbinding activity.

Another embodiment for generating effective peptide analogs involves the substitution of residues that have an adverse impact on peptide stability or solubility in, *e.g.*, a liquid environment. This substitution may occur at any position of the peptide epitope. For example, a cysteine (C) can be substituted out in favor of α -amino butyric acid. Due to its chemical nature, cysteine has the propensity to form disulfide bridges and sufficiently alter the peptide structurally so as to reduce binding capacity. Substituting α -amino butyric acid for C not only alleviates this problem, but actually improves binding and crossbinding capability in certain instances (*see, e.g.*, the review by Sette *et al.*, In: Persistent Viral Infections, Eds. R. Ahmed and I. Chen, John Wiley & Sons, England, 1999). Substitution of cysteine with α -amino butyric acid may occur at any residue of a peptide epitope, *i.e.* at either anchor or non-anchor positions.

Representative analog peptides are set forth in Table XXII. The Table indicates the length and sequence of the analog peptide as well as the motif or supermotif, if appropriate. The information in the "Fixed Nomenclature" column indicates the residues substituted at the indicated position numbers for the respective analog.

IV.G. Computer Screening of Protein Sequences from Disease-Related Antigens for Supermotif- or Motif-Bearing Peptides

In order to identify supermotif- or motif-bearing epitopes in a target antigen, a native protein sequence, *e.g.*, a tumor-associated antigen, or sequences from an infectious organism, or a donor tissue for transplantation, is screened using a means for computing, such as an intellectual calculation or a computer, to determine the presence of a supermotif or motif within the sequence. The information obtained from the analysis of native peptide can be used directly to evaluate the status of the native peptide or may be utilized subsequently to generate the peptide epitope.

Computer programs that allow the rapid screening of protein sequences for the occurrence of the subject supermotifs or motifs are encompassed by the present invention; as are programs that permit the generation of analog peptides. These programs are implemented to analyze any identified amino acid sequence or operate on an unknown sequence and simultaneously determine the sequence and identify motif-bearing epitopes thereof; analogs can be simultaneously determined as well. Generally, the identified sequences will be from a pathogenic organism or a tumor-associated peptide. For

example, the target molecules considered herein include, without limitation, the core, S, E1, NS1/E2, NS2, NS3, NS4, and NS5 regions of HCV.

In cases where the sequence of multiple variants of the same target protein are available, peptides may also be selected on the basis of their conservancy. A presently preferred criterion for conservancy defines that the entire sequence of an HLA class I binding peptide or the entire 9-mer core of a class II binding peptide, be totally (*i.e.*, 100%) conserved in at least 79% of the sequences evaluated for a specific protein. This definition of conservancy has been employed herein; although, as appreciated by those in the art, lower or higher degrees of conservancy can be employed as appropriate for a given antigenic target.

It is important that the selection criteria utilized for prediction of peptide binding are as accurate as possible, to correlate most efficiently with actual binding. Prediction of peptides that bind, for example, to HLA-A*0201, on the basis of the presence of the appropriate primary anchors, is positive at about a 30% rate (see, *e.g.*, Ruppert, J. *et al. Cell* 74:929, 1993). However, by extensively analyzing peptide-HLA binding data disclosed herein, data in related patent applications, and data in the art, the present inventors have developed a number of allele-specific polynomial algorithms that dramatically increase the predictive value over identification on the basis of the presence of primary anchor residues alone. These algorithms take into account not only the presence or absence of primary anchors, but also consider the positive or deleterious presence of secondary anchor residues (to account for the impact of different amino acids at different positions). The algorithms are essentially based on the premise that the overall affinity (or ΔG) of peptide-HLA interactions can be approximated as a linear polynomial function of the type:

$$\Delta G = a_{1i} \times a_{2i} \times a_{3i} \dots \times a_{ni}$$

where a_{ji} is a coefficient that represents the effect of the presence of a given amino acid (j) at a given position (i) along the sequence of a peptide of n amino acids. An important assumption of this method is that the effects at each position are essentially independent of each other. This assumption is justified by studies that demonstrated that peptides are bound to HLA molecules and recognized by T cells in essentially an extended conformation. Derivation of specific algorithm coefficients has been described, for example, in Gulukota, K. *et al.*, *J. Mol. Biol.* 267:1258, 1997.

Additional methods to identify preferred peptide sequences, which also make use of specific motifs, include the use of neural networks and molecular modeling programs (see, e.g., Milik *et al.*, *Nature Biotechnology* 16:753, 1998; Altuvia *et al.*, *Hum. Immunol.* 58:1, 1997; Altuvia *et al.*, *J. Mol. Biol.* 249:244, 1995; Buus, S. *Curr. Opin. Immunol.* 11:209-213, 1999; Brusic, V. *et al.*, *Bioinformatics* 14:121-130, 1998; Parker *et al.*, *J. Immunol.* 152:163, 1993; Meister *et al.*, *Vaccine* 13:581, 1995; Hammer *et al.*, *J. Exp. Med.* 180:2353, 1994; Sturniolo *et al.*, *Nature Biotechnol.* 17:555 1999).

For example, it has been shown that in sets of A*0201 motif-bearing peptides containing at least one preferred secondary anchor residue while avoiding the presence of any deleterious secondary anchor residues, 69% of the peptides will bind A*0201 with an IC₅₀ less than 500 nM (Ruppert, J. *et al. Cell* 74:929, 1993). These algorithms are also flexible in that cut-off scores may be adjusted to select sets of peptides with greater or lower predicted binding properties, as desired.

In utilizing computer screening to identify peptide epitopes, a protein sequence or translated sequence may be analyzed using software developed to search for motifs, for example the "FINDPATTERNS" program (Devereux, *et al. Nucl. Acids Res.* 12:387-395, 1984) or MotifSearch 1.4 software program (D. Brown, San Diego, CA) to identify potential peptide sequences containing appropriate HLA binding motifs. The identified peptides can be scored using customized polynomial algorithms to predict their capacity to bind specific HLA class I or class II alleles. As appreciated by one of ordinary skill in the art, a large array of computer programming software and hardware options are available in the relevant art which can be employed to implement the motifs of the invention in order to evaluate (e.g., without limitation, to identify epitopes, identify epitope concentration per peptide length, or to generate analogs) known or unknown peptide sequences.

In accordance with the procedures described above, HCV peptide epitopes and analogs thereof that are able to bind HLA supertype groups or allele-specific HLA molecules have been identified (Tables VII-XX; Table XXII).

IV.H. Preparation of Peptide Epitopes

Peptides in accordance with the invention can be prepared synthetically, by recombinant DNA technology or chemical synthesis, or from natural sources such as native tumors or pathogenic organisms. Peptide epitopes may be synthesized individually or as polyepitopic peptides. Although the peptide will preferably be substantially free of

other naturally occurring host cell proteins and fragments thereof, in some embodiments the peptides may be synthetically conjugated to native fragments or particles.

The peptides in accordance with the invention can be a variety of lengths, and either in their neutral (uncharged) forms or in forms which are salts. The peptides in accordance with the invention are either free of modifications such as glycosylation, side chain oxidation, or phosphorylation; or they contain these modifications, subject to the condition that modifications do not destroy the biological activity of the peptides as described herein.

The peptides of the invention can be prepared in a wide variety of ways. For the preferred relatively short size, the peptides can be synthesized in solution or on a solid support in accordance with conventional techniques. Various automatic synthesizers are commercially available and can be used in accordance with known protocols. (See, for example, Stewart & Young, SOLID PHASE PEPTIDE SYNTHESIS, 2D. ED., Pierce Chemical Co., 1984). Further, individual peptide epitopes can be joined using chemical ligation to produce larger peptides that are still within the bounds of the invention.

Alternatively, recombinant DNA technology can be employed wherein a nucleotide sequence which encodes an immunogenic peptide of interest is inserted into an expression vector, transformed or transfected into an appropriate host cell and cultivated under conditions suitable for expression. These procedures are generally known in the art, as described generally in Sambrook *et al.*, MOLECULAR CLONING, A LABORATORY MANUAL, Cold Spring Harbor Press, Cold Spring Harbor, New York (1989). Thus, recombinant polypeptides which comprise one or more peptide sequences of the invention can be used to present the appropriate T cell epitope.

The nucleotide coding sequence for peptide epitopes of the preferred lengths contemplated herein can be synthesized by chemical techniques, for example, the phosphotriester method of Matteucci, *et al.*, *J. Am. Chem. Soc.* 103:3185 (1981). Peptide analogs can be made simply by substituting the appropriate and desired nucleic acid base(s) for those that encode the native peptide sequence; exemplary nucleic acid substitutions are those that encode an amino acid defined by the motifs/super motifs herein. The coding sequence can then be provided with appropriate linkers and ligated into expression vectors commonly available in the art, and the vectors used to transform suitable hosts to produce the desired fusion protein. A number of such vectors and suitable host systems are now available. For expression of the fusion proteins, the coding sequence will be provided with operably linked start and stop codons, promoter and

terminator regions and usually a replication system to provide an expression vector for expression in the desired cellular host. For example, promoter sequences compatible with bacterial hosts are provided in plasmids containing convenient restriction sites for insertion of the desired coding sequence. The resulting expression vectors are
5 transformed into suitable bacterial hosts. Of course, yeast, insect or mammalian cell hosts may also be used, employing suitable vectors and control sequences.

It is often preferable that the peptide epitope be as small as possible while still maintaining substantially all of the immunologic activity of the native protein. When possible, it may be desirable to optimize HLA class I binding peptide epitopes of the
10 invention to a length of about 8 to about 13 amino acid residues, preferably 9 to 10. HLA class II binding peptide epitopes may be optimized to a length of about 6 to about 30 amino acids in length, preferably to between about 13 and about 20 residues. Preferably, the peptide epitopes are commensurate in size with endogenously processed pathogen-derived peptides or tumor cell peptides that are bound to the relevant HLA molecules,
15 however, the identification and preparation of peptides of other lengths can also be carried out using the techniques described herein.

In alternative embodiments, peptides of the invention can be linked as a polyepitopic peptide, or as a minigene that encodes a polyepitopic peptide.

In another embodiment, it is preferred to identify native peptide regions that
20 contain a high concentration of class I and/or class II epitopes. Such a sequence is generally selected on the basis that it contains the greatest number of epitopes per amino acid length. It is to be appreciated that epitopes can be present in a frame-shifted manner, e.g. a 10 amino acid long peptide could contain two 9 amino acid long epitopes and one 10 amino acid long epitope; upon intracellular processing, each epitope can be exposed
25 and bound by an HLA molecule upon administration of such a peptide. This larger, preferably multi-epitopic, peptide can be generated synthetically, recombinantly, or via cleavage from the native source.

IV.I. Assays to Detect T-Cell Responses

30 Once HLA binding peptides are identified, they can be tested for the ability to elicit a T-cell response. The preparation and evaluation of motif-bearing peptides are described in PCT publications WO 94/20127 and WO 94/03205. Briefly, peptides comprising epitopes from a particular antigen are synthesized and tested for their ability to bind to the appropriate HLA proteins. These assays may involve evaluating the

binding of a peptide of the invention to purified HLA class I molecules in relation to the binding of a radioiodinated reference peptide. Alternatively, cells expressing empty class I molecules (*i.e.* lacking peptide therein) may be evaluated for peptide binding by immunofluorescent staining and flow microfluorimetry. Other assays that may be used to evaluate peptide binding include peptide-dependent class I assembly assays and/or the inhibition of CTL recognition by peptide competition. Those peptides that bind to the class I molecule, typically with an affinity of 500 nM or less, are further evaluated for their ability to serve as targets for CTLs derived from infected or immunized individuals, as well as for their capacity to induce primary *in vitro* or *in vivo* CTL responses that can give rise to CTL populations capable of reacting with selected target cells associated with a disease. Corresponding assays are used for evaluation of HLA class II binding peptides. HLA class II motif-bearing peptides that are shown to bind, typically at an affinity of 1000 nM or less, are further evaluated for the ability to stimulate HTL responses.

Conventional assays utilized to detect T cell responses include proliferation assays, lymphokine secretion assays, direct cytotoxicity assays, and limiting dilution assays. For example, antigen-presenting cells that have been incubated with a peptide can be assayed for the ability to induce CTL responses in responder cell populations. Antigen-presenting cells can be normal cells such as peripheral blood mononuclear cells or dendritic cells. Alternatively, mutant non-human mammalian cell lines that are deficient in their ability to load class I molecules with internally processed peptides and that have been transfected with the appropriate human class I gene, may be used to test for the capacity of the peptide to induce *in vitro* primary CTL responses.

Peripheral blood mononuclear cells (PBMCs) may be used as the responder cell source of CTL precursors. The appropriate antigen-presenting cells are incubated with peptide, after which the peptide-loaded antigen-presenting cells are then incubated with the responder cell population under optimized culture conditions. Positive CTL activation can be determined by assaying the culture for the presence of CTLs that kill radio-labeled target cells, both specific peptide-pulsed targets as well as target cells expressing endogenously processed forms of the antigen from which the peptide sequence was derived.

More recently, a method has been devised which allows direct quantification of antigen-specific T cells by staining with Fluorescein-labelled HLA tetrameric complexes (Altman, J. D. *et al.*, *Proc. Natl. Acad. Sci. USA* 90:10330, 1993; Altman, J. D. *et al.*, *Science* 274:94, 1996). Other relatively recent technical developments include staining

for intracellular lymphokines, and interferon release assays or ELISPOT assays.

Tetramer staining, intracellular lymphokine staining and ELISPOT assays all appear to be at least 10-fold more sensitive than more conventional assays (Lalvani, A. *et al.*, *J. Exp. Med.* 186:859, 1997; Dunbar, P. R. *et al.*, *Curr. Biol.* 8:413, 1998; Murali-Krishna, K. *et al.*, *Immunity* 8:177, 1998).

HTL activation may also be assessed using such techniques known to those in the art such as T cell proliferation and secretion of lymphokines, *e.g.* IL-2 (*see, e.g.* Alexander *et al.*, *Immunity* 1:751-761, 1994).

Alternatively, immunization of HLA transgenic mice can be used to determine immunogenicity of peptide epitopes. Several transgenic mouse models including mice with human A2.1, A11 (which can additionally be used to analyze HLA-A3 epitopes), and B7 alleles have been characterized and others (*e.g.*, transgenic mice for HLA-A1 and A24) are being developed. HLA-DR1 and HLA-DR3 mouse models have also been developed. Additional transgenic mouse models with other HLA alleles may be generated as necessary. Mice may be immunized with peptides emulsified in Incomplete Freund's Adjuvant and the resulting T cells tested for their capacity to recognize peptide-pulsed target cells and target cells transfected with appropriate genes. CTL responses may be analyzed using cytotoxicity assays described above. Similarly, HTL responses may be analyzed using such assays as T cell proliferation or secretion of lymphokines.

Exemplary immunogenic peptide epitopes are set out in Table XXIII.

IV.J. Use of Peptide Epitopes as Diagnostic Agents and for Evaluating Immune Responses

In one embodiment of the invention, HLA class I and class II binding peptides as described herein can be used as reagents to evaluate an immune response. The immune response to be evaluated can be induced by using as an immunogen any agent that may result in the production of antigen-specific CTLs or HTLs that recognize and bind to the peptide epitope(s) to be employed as the reagent. The peptide reagent need not be used as the immunogen. Assay systems that can be used for such an analysis include relatively recent technical developments such as tetramers, staining for intracellular lymphokines and interferon release assays, or ELISPOT assays.

For example, a peptide of the invention may be used in a tetramer staining assay to assess peripheral blood mononuclear cells for the presence of antigen-specific CTLs following exposure to a tumor cell antigen or an immunogen. The HLA-tetrameric

complex is used to directly visualize antigen-specific CTLs (*see, e.g., Ogg et al., Science* 279:2103-2106, 1998; and Altman *et al., Science* 174:94-96, 1996) and determine the frequency of the antigen-specific CTL population in a sample of peripheral blood mononuclear cells. A tetramer reagent using a peptide of the invention may be generated
5 as follows: A peptide that binds to an HLA molecule is refolded in the presence of the corresponding HLA heavy chain and β_2 -microglobulin to generate a trimolecular complex. The complex is biotinylated at the carboxyl terminal end of the heavy chain at a site that was previously engineered into the protein. Tetramer formation is then induced by the addition of streptavidin. By means of fluorescently labeled streptavidin, the
10 tetramer can be used to stain antigen-specific cells. The cells may then be identified, for example, by flow cytometry. Such an analysis may be used for diagnostic or prognostic purposes. Cells identified by the procedure can also be used for therapeutic purposes.

Peptides of the invention may also be used as reagents to evaluate immune recall responses. (*see, e.g., Bertoni et al., J. Clin. Invest.* 100:503-513, 1997 and Penna *et al., J.*
15 *Exp. Med.* 174:1565-1570, 1991.) For example, patient PBMC samples from individuals with HCV infection may be analyzed for the presence of antigen-specific CTLs or HTLs using specific peptides. A blood sample containing mononuclear cells may be evaluated by cultivating the PBMCs and stimulating the cells with a peptide of the invention. After an appropriate cultivation period, the expanded cell population may be analyzed, for
20 example, for cytotoxic activity (CTL) or for HTL activity.

The peptides may also be used as reagents to evaluate the efficacy of a vaccine. PBMCs obtained from a patient vaccinated with an immunogen may be analyzed using, for example, either of the methods described above. The patient is HLA typed, and peptide epitope reagents that recognize the allele-specific molecules present in that
25 patient are selected for the analysis. The immunogenicity of the vaccine is indicated by the presence of epitope-specific CTLs and/or HTLs in the PBMC sample.

The peptides of the invention may also be used to make antibodies, using techniques well known in the art (*see, e.g. CURRENT PROTOCOLS IN IMMUNOLOGY*, Wiley/Greene, NY; and *Antibodies A Laboratory Manual*, Harlow and Lane, Cold Spring
30 Harbor Laboratory Press, 1989), which may be useful as reagents to diagnose or monitor cancer. Such antibodies include those that recognize a peptide in the context of an HLA molecule, *i.e.*, antibodies that bind to a peptide-MHC complex.

IV.K. Vaccine Compositions

- Vaccines and methods of preparing vaccines that contain an immunogenically effective amount of one or more peptides as described herein are further embodiments of the invention. Once appropriately immunogenic epitopes have been defined, they can be sorted and delivered by various means, herein referred to as "vaccine" compositions. Such vaccine compositions can include, for example, lipopeptides (*e.g.*, Vitiello, A. *et al.*, *J. Clin. Invest.* 95:341, 1995), peptide compositions encapsulated in poly(DL-lactide-co-glycolide) ("PLG") microspheres (*see, e.g.*, Eldridge, *et al.*, *Molec. Immunol.* 28:287-294, 1991; Alonso *et al.*, *Vaccine* 12:299-306, 1994; Jones *et al.*, *Vaccine* 13:675-681, 1995), peptide compositions contained in immune stimulating complexes (ISCOMS) (*see, e.g.*, Takahashi *et al.*, *Nature* 344:873-875, 1990; Hu *et al.*, *Clin Exp Immunol.* 113:235-243, 1998), multiple antigen peptide systems (MAPs) (*see e.g.*, Tam, J. P., *Proc. Natl. Acad. Sci. U.S.A.* 85:5409-5413, 1988; Tam, J.P., *J. Immunol. Methods* 196:17-32, 1996), viral delivery vectors (Perkus, M. E. *et al.*, In: *Concepts in vaccine development*, Kaufmann, S. H. E., ed., p. 379, 1996; Chakrabarti, S. *et al.*, *Nature* 320:535, 1986; Hu, S. L. *et al.*, *Nature* 320:537, 1986; Kieny, M.-P. *et al.*, *AIDS Bio/Technology* 4:790, 1986; Top, F. H. *et al.*, *J. Infect. Dis.* 124:148, 1971; Chanda, P. K. *et al.*, *Virology* 175:535, 1990), particles of viral or synthetic origin (*e.g.*, Kofler, N. *et al.*, *J. Immunol. Methods* 192:25, 1996; Eldridge, J. H. *et al.*, *Sem. Hematol.* 30:16, 1993; Falo, L. D., Jr. *et al.*, *Nature Med.* 7:649, 1995), adjuvants (Warren, H. S., Vogel, F. R., and Chedid, L. A. *Annu. Rev. Immunol.* 4:369, 1986; Gupta, R. K. *et al.*, *Vaccine* 11:293, 1993), liposomes (Reddy, R. *et al.*, *J. Immunol.* 148:1585, 1992; Rock, K. L., *Immunol. Today* 17:131, 1996), or, naked or particle absorbed cDNA (Ulmer, J. B. *et al.*, *Science* 259:1745, 1993; Robinson, H. L., Hunt, L. A., and Webster, R. G., *Vaccine* 11:957, 1993; Shiver, J. W. *et al.*, In: *Concepts in vaccine development*, Kaufmann, S. H. E., ed., p. 423, 1996; Cease, K. B., and Berzofsky, J. A., *Annu. Rev. Immunol.* 12:923, 1994 and Eldridge, J. H. *et al.*, *Sem. Hematol.* 30:16, 1993). Toxin-targeted delivery technologies, also known as receptor mediated targeting, such as those of Avant Immunotherapeutics, Inc. (Needham, Massachusetts) may also be used.
- Vaccines of the invention include nucleic acid-mediated modalities. DNA or RNA encoding one or more of the peptides of the invention can also be administered to a patient. This approach is described, for instance, in Wolff *et. al.*, *Science* 247:1465 (1990) as well as U.S. Patent Nos. 5,580,859; 5,589,466; 5,804,566; 5,739,118; 5,736,524; 5,679,647; WO 98/04720; and in more detail below. Examples of DNA-based

delivery technologies include "naked DNA", facilitated (bupivacaine, polymers, peptide-mediated) delivery, cationic lipid complexes, and particle-mediated ("gene gun") or pressure-mediated delivery (*see, e.g.*, U.S. Patent No. 5,922,687).

For therapeutic or prophylactic immunization purposes, the peptides of the invention can also be expressed by viral or bacterial vectors. Examples of expression vectors include attenuated viral hosts, such as vaccinia or fowlpox. As an example of this approach, vaccinia virus is used as a vector to express nucleotide sequences that encode the peptides of the invention. Upon introduction into a host bearing a tumor, the recombinant vaccinia virus expresses the immunogenic peptide, and thereby elicits a host CTL and/or HTL response. Vaccinia vectors and methods useful in immunization protocols are described in, *e.g.*, U.S. Patent No. 4,722,848. Another vector is BCG (Bacille Calmette Guerin). BCG vectors are described in Stover *et al.*, *Nature* 351:456-460 (1991). A wide variety of other vectors useful for therapeutic administration or immunization of the peptides of the invention, *e.g.* adeno and adeno-associated virus vectors, retroviral vectors, *Salmonella typhi* vectors, detoxified anthrax toxin vectors, and the like, will be apparent to those skilled in the art from the description herein.

Furthermore, vaccines in accordance with the invention encompass compositions of one or more of the claimed peptide(s). A peptide can be present in a vaccine individually. Alternatively, the peptide can exist as a homopolymer comprising multiple copies of the same peptide, or as a heteropolymer of various peptides. Polymers have the advantage of increased immunological reaction and, where different peptide epitopes are used to make up the polymer, the additional ability to induce antibodies and/or CTLs that react with different antigenic determinants of the pathogenic organism or tumor-related peptide targeted for an immune response. The composition can be a naturally occurring region of an antigen or can be prepared, *e.g.*, recombinantly or by chemical synthesis.

Carriers that can be used with vaccines of the invention are well known in the art, and include, *e.g.*, thyroglobulin, albumins such as human serum albumin, tetanus toxoid, polyamino acids such as poly L-lysine, poly L-glutamic acid, influenza, hepatitis B virus core protein, and the like. The vaccines can contain a physiologically tolerable (*i.e.*, acceptable) diluent such as water, or saline, preferably phosphate buffered saline. The vaccines also typically include an adjuvant. Adjuvants such as incomplete Freund's adjuvant, aluminum phosphate, aluminum hydroxide, or alum are examples of materials well known in the art. Additionally, as disclosed herein, CTL responses can be primed by

conjugating peptides of the invention to lipids, such as tripalmitoyl-S-glycerylcysteinylserine (P₃CSS).

Upon immunization with a peptide composition in accordance with the invention, via injection, aerosol, oral, transdermal, transmucosal, intrapleural, intrathecal, or other
5 suitable routes, the immune system of the host responds to the vaccine by producing large amounts of CTLs and/or HTLs specific for the desired antigen. Consequently, the host becomes at least partially immune to later infection, or at least partially resistant to developing an ongoing chronic infection, or derives at least some therapeutic benefit when the antigen was tumor-associated.

10 In some embodiments it may be desirable to combine the class I peptide components with components that induce or facilitate neutralizing antibody responses to the target antigen of interest, particularly to viral envelope antigens. A preferred embodiment of such a composition comprises class I and class II epitopes in accordance with the invention. An alternative embodiment of such a composition comprises a class I
15 and/or class II epitope in accordance with the invention, along with a PADRE™ (Epimmune, San Diego, CA) molecule (described, for example, in U.S. Patent Number 5,736,142).

A vaccine of the invention can also include antigen-presenting cells, such as dendritic cells, as a vehicle to present peptides of the invention. Vaccine compositions
20 can be created *in vitro*, following dendritic cell mobilization and harvesting, whereby loading of dendritic cells occurs *in vitro*. For example, dendritic cells are transfected, e.g., with a minigene in accordance with the invention. The dendritic cell can then be administered to a patient to elicit immune responses *in vivo*.

Antigenic peptides are used to elicit a CTL and/or HTL response *ex vivo*, as well.
25 The resulting CTL or HTL cells, can be used to treat tumors in patients that do not respond to other conventional forms of therapy, or will not respond to a therapeutic vaccine peptide or nucleic acid in accordance with the invention. *Ex vivo* CTL or HTL responses to a particular tumor-associated antigen are induced by incubating in tissue culture the patient's, or genetically compatible, CTL or HTL precursor cells together with
30 a source of antigen-presenting cells (APC), such as dendritic cells, and the appropriate immunogenic peptide. After an appropriate incubation time (typically about 7-28 days), in which the precursor cells are activated and expanded into effector cells, the cells are infused back into the patient, where they will destroy (CTL) or facilitate destruction

(HTL) of their specific target cell (an infected cell or a tumor cell). Transfected dendritic cells may also be used as antigen presenting cells.

The vaccine compositions of the invention can also be used in combination with antiviral drugs such as interferon- α , or other treatments for viral infection.

5 Preferably, the following principles are utilized when selecting an array of epitopes for inclusion in a polyepitopic composition for use in a vaccine, or for selecting discrete epitopes to be included in a vaccine and/or to be encoded by nucleic acids such as a minigene. It is preferred that each of the following principles are balanced in order to make the selection. The multiple epitopes to be incorporated in a given vaccine
10 composition may be, but need not be, contiguous in sequence in the native antigen from which the epitopes are derived.

 Preferably, the following principles are utilized when selecting an array of epitopes for inclusion in a polyepitopic composition for use in a vaccine, or for selecting discrete epitopes to be included in a vaccine and/or to be encoded by nucleic acids such as
15 a minigene. Exemplary epitopes that may be utilized in a vaccine to treat or prevent HCV infection are set out in Tables XXVI-XXIX, and Table XXXII. It is preferred that each of the following principles are balanced in order to make the selection.

 1.) Epitopes are selected which, upon administration, mimic immune responses that have been observed to be correlated with HCV clearance. For HLA Class I
20 this includes 3-4 epitopes that come from at least one antigen of HCV. For HLA Class II a similar rationale is employed; again 3-4 epitopes are selected from at least one HCV antigen (*see e.g.*, Rosenberg *et al.*, *Science* 278:1447-1450).

 2.) Epitopes are selected that have the requisite binding affinity established to be correlated with immunogenicity: for HLA Class I an IC_{50} of 500 nM or less, or for
25 Class II an IC_{50} of 1000 nM or less.

 3.) Sufficient supermotif bearing-peptides, or a sufficient array of allele-specific motif-bearing peptides, are selected to give broad population coverage. For example, it is preferable to have at least 80% population coverage. A Monte Carlo analysis, a statistical evaluation known in the art, can be employed to assess the breadth,
30 or redundancy of, population coverage.

 4.) When selecting epitopes from cancer-related antigens it is often preferred to select analogs because the patient may have developed tolerance to the native epitope.

When selecting epitopes for infectious disease-related antigens it is preferable to select either native or analoged epitopes.

5.) Of particular relevance are epitopes referred to as "nested epitopes."

Nested epitopes occur where at least two epitopes overlap in a given peptide sequence. A

5 nested peptide sequence can comprise both HLA class I and HLA class II epitopes.

When providing nested epitopes, it is preferable to provide a sequence that has the greatest number of epitopes per provided sequence. Preferably, one avoids providing a peptide that is any longer than the amino terminus of the amino terminal epitope and the carboxyl terminus of the carboxyl terminal epitope in the peptide. When providing a
10 longer peptide sequence, such as a sequence comprising nested epitopes, it is important to screen the sequence in order to insure that it does not have pathological or other deleterious biological properties.

6.) If a polyepitopic protein is created, or when creating a minigene, an objective is to generate the smallest peptide that encompasses the epitopes of interest.

15 This principle is similar, if not the same as that employed when selecting a peptide comprising nested epitopes. However, with an artificial polyepitopic peptide, the size minimization objective is balanced against the need to integrate any spacer sequences between epitopes in the polyepitopic protein. Spacer amino acid residues can be introduced to avoid junctional epitopes (an epitope recognized by the immune system, not
20 present in the target antigen, and only created by the man-made juxtaposition of epitopes), or to facilitate cleavage between epitopes and thereby enhance epitope presentation. Junctional epitopes are generally to be avoided because the recipient may generate an immune response to that non-native epitope. Of particular concern is a junctional epitope that is a "dominant epitope." A dominant epitope may lead to such a
25 zealous response that immune responses to other epitopes are diminished or suppressed.

Examples of polyepitopic vaccine compositions designed based on the above criteria can include epitopes from the core, S, E1, NS1/E2, NS2, NS3, NS4, and NS5 domains of the HCV polyprotein. These regions encompass the following amino acid sequences using numbering relative to the prototype HCV-1 strain (Genbank accession
30 number M62321; *see, e.g.*, US Patent Nos. 5,683,864 and 5,670,153): C domain (amino acids 1-120); S (amino acids 120-400); NS3 (amino acids 1050-1640); NS4 (amino acids 1640-2000); NS5 (amino acids 2000-3011); and envelop proteins, E1 and E2/NS1, encompassing amino acids 192-750. Amino acids 750 to 1050 are designated as domain X as applied to the present invention. As appreciated by one of ordinary skill in the art,

the designation of the amino acid range for each domain may diverge to some extent from that of HCV-1 depending on the strain of HCV. One of ordinary skill in the art, when looking at an HCV polyprotein sequence, would readily be able to determine the domain boundaries.

- 5 Specific embodiments of the polyepitopic compositions of the present invention include a pharmaceutical composition comprising a pharmaceutically acceptable carrier and combination of motif-bearing peptides that are immunologically cross-reactive with peptides of HCV-1, wherein at least one of the peptides bears a motif of Table Ia, and further wherein the combination of motif-bearing peptides consists of: a) one or more
- 10 peptides comprising at least 8 amino acids from an HCV C domain; b) one or more peptides comprising at least 8 amino acids of a further domain selected from the group consisting of: an S domain, an NS3 domain, an NS4 domain, or an NS5 domain, and; c) optionally, one or more motif-bearing peptides from one or more additional HCV domains with a *proviso* that an additional domain is not a further domain listed in "b".
- 15 Preferably, such a pharmaceutical composition may additionally comprise one or more distinct HCV motif-bearing peptide(s) comprising at least 8 amino acids of an X domain or, alternatively, the composition may further comprise additional HCV motif-bearing peptide(s) that are from an envelope domain, the envelope domain peptide(s) consisting of one or more copies of a single HCV peptide comprising at least 8 amino acids of an
- 20 envelope domain.

- In another embodiment, the polyepitopic pharmaceutical composition may comprise a pharmaceutically acceptable carrier and combination of motif-bearing peptides that are immunologically cross-reactive with HCV-1 peptides, the peptides from multiple domains of HCV, wherein at least one of the peptides bears a motif of Table Ia,
- 25 and wherein the combination of motif-bearing peptides consists essentially of: a) one or more peptides comprising at least 8 amino acids from a C domain; and, b) one or more peptides comprising at least 8 amino acids from an S, NS3, NS4, or NS5 domain, and, one HCV peptide comprising at least 8 amino acids of an envelope domain. Such a composition may further comprise one or more HCV motif-bearing peptides comprising
- 30 at least 8 amino acids of an X domain.

 Alternatively, a pharmaceutical composition of the invention may comprise: a) a pharmaceutically acceptable carrier; and, b) a combination of one or more motif-bearing peptides of at least 8 amino acids derived from one or more hepatitis C virus (HCV) domains, wherein said peptides are cross-reactive with peptides of HCV-1, with a *proviso*

that the combination does not include a peptide of at least 8 amino acids from an HCV C domain, and wherein at least one of the peptides bears a motif of Table Ia, said domains selected from the group consisting of: an S domain; an NS3 domain; an NS4 domain; an NS5 domain; and, an X domain. Such a composition may additionally comprise motif-
5 bearing HCV envelope peptide(s) consisting of one or more copies of a single HCV peptide comprising at least 8 amino acids of an envelope domain.

Lastly, an embodiment of the invention may comprise a pharmaceutical composition comprising a pharmaceutically acceptable carrier and combination of two or more motif-bearing peptides from a single domain of an HCV-1 strain, said peptides
10 immunologically cross-reactive with peptides of an HCV-1 antigen, wherein at least one of the peptides bears a motif of Table Ia, and the peptides are derived from HCV, and the HCV domain is selected from the group consisting of: a C domain; an S domain; an NS3 domain; an NS4 domain; an NS5 domain; an X domain; or, an envelope domain from a single HCV strain, with a *proviso* that the envelope domain is other than a variable
15 envelope domain.

In the embodiments set forth, "peptides immunologically cross-reactive with HCV-1" refers to peptides that are bound by the same antibody; "derived from" refers to a fragment or subsequence and conservatively modified variants thereof.

20 IV.K.1. Minigene Vaccines

A number of different approaches are available which allow simultaneous delivery of multiple epitopes. Nucleic acids encoding the peptides of the invention are a particularly useful embodiment of the invention. Epitopes for inclusion in a minigene are preferably selected according to the guidelines set forth in the previous section. A
25 preferred means of administering nucleic acids encoding the peptides of the invention uses minigene constructs encoding a peptide comprising one or multiple epitopes of the invention.

The use of multi-epitope minigenes is described below and in, *e.g.*, co-pending application U.S.S.N. 09/311,784; An, L. and Whitton, J. L., *J. Virol.* 71:2292, 1997;
30 Thomson, S. A. *et al.*, *J. Immunol.* 157:822, 1996; Whitton, J. L. *et al.*, *J. Virol.* 67:348, 1993; Hanke, R. *et al.*, *Vaccine* 16:426, 1998. For example, a multi-epitope DNA plasmid encoding supermotif- and/or motif-bearing HCV epitopes derived from multiple regions of the HCV polyprotein sequence, the PADRE™ universal helper T cell epitope (or

multiple HTL epitopes from HCV), and an endoplasmic reticulum-translocating signal sequence can be engineered.

The immunogenicity of a multi-epitopic minigene can be tested in transgenic mice to evaluate the magnitude of CTL induction responses against the epitopes tested.

5 Further, the immunogenicity of DNA-encoded epitopes *in vivo* can be correlated with the *in vitro* responses of specific CTL lines against target cells transfected with the DNA plasmid. Thus, these experiments can show that the minigene serves to both: 1.) generate a CTL response and 2.) that the induced CTLs recognized cells expressing the encoded epitopes.

10 For example, to create a DNA sequence encoding the selected epitopes (minigene) for expression in human cells, the amino acid sequences of the epitopes may be reverse translated. A human codon usage table can be used to guide the codon choice for each amino acid. These epitope-encoding DNA sequences may be directly adjoined, so that when translated, a continuous polypeptide sequence is created. To optimize expression and/or immunogenicity, additional elements can be incorporated into the minigene design. Examples of amino acid sequences that can be reverse translated and included in the minigene sequence include: HLA class I epitopes, HLA class II epitopes, a ubiquitination signal sequence, and/or an endoplasmic reticulum targeting signal. In addition, HLA presentation of CTL and HTL epitopes may be improved by including synthetic (*e.g.* poly-alanine) or naturally-occurring flanking sequences adjacent to the CTL or HTL epitopes; these larger peptides comprising the epitope(s) are within the scope of the invention.

25 The minigene sequence may be converted to DNA by assembling oligonucleotides that encode the plus and minus strands of the minigene. Overlapping oligonucleotides (30-100 bases long) may be synthesized, phosphorylated, purified and annealed under appropriate conditions using well known techniques. The ends of the oligonucleotides can be joined, for example, using T4 DNA ligase. This synthetic minigene, encoding the epitope polypeptide, can then be cloned into a desired expression vector.

30 Standard regulatory sequences well known to those of skill in the art are preferably included in the vector to ensure expression in the target cells. Several vector elements are desirable: a promoter with a down-stream cloning site for minigene insertion; a polyadenylation signal for efficient transcription termination; an *E. coli* origin of replication; and an *E. coli* selectable marker (*e.g.* ampicillin or kanamycin resistance). Numerous promoters can be used for this purpose, *e.g.*, the human cytomegalovirus

(hCMV) promoter. See, *e.g.*, U.S. Patent Nos. 5,580,859 and 5,589,466 for other suitable promoter sequences.

Additional vector modifications may be desired to optimize minigene expression and immunogenicity. In some cases, introns are required for efficient gene expression, and one or more synthetic or naturally-occurring introns could be incorporated into the transcribed region of the minigene. The inclusion of mRNA stabilization sequences and sequences for replication in mammalian cells may also be considered for increasing minigene expression.

Once an expression vector is selected, the minigene is cloned into the polylinker region downstream of the promoter. This plasmid is transformed into an appropriate *E. coli* strain, and DNA is prepared using standard techniques. The orientation and DNA sequence of the minigene, as well as all other elements included in the vector, are confirmed using restriction mapping and DNA sequence analysis. Bacterial cells harboring the correct plasmid can be stored as a master cell bank and a working cell bank.

In addition, immunostimulatory sequences (ISSs or CpGs) appear to play a role in the immunogenicity of DNA vaccines. These sequences may be included in the vector, outside the minigene coding sequence, if desired to enhance immunogenicity.

In some embodiments, a bi-cistronic expression vector which allows production of both the minigene-encoded epitopes and a second protein (included to enhance or decrease immunogenicity) can be used. Examples of proteins or polypeptides that could beneficially enhance the immune response if co-expressed include cytokines (*e.g.*, IL-2, IL-12, GM-CSF), cytokine-inducing molecules (*e.g.*, LeIF), costimulatory molecules, or for HTL responses, pan-DR binding proteins (PADRE™, Epimmune, San Diego, CA). Helper (HTL) epitopes can be joined to intracellular targeting signals and expressed separately from expressed CTL epitopes; this allows direction of the HTL epitopes to a cell compartment different than that of the CTL epitopes. If required, this could facilitate more efficient entry of HTL epitopes into the HLA class II pathway, thereby improving HTL induction. In contrast to HTL or CTL induction, specifically decreasing the immune response by co-expression of immunosuppressive molecules (*e.g.* TGF-β) may be beneficial in certain diseases.

Therapeutic quantities of plasmid DNA can be produced for example, by fermentation in *E. coli*, followed by purification. Aliquots from the working cell bank are used to inoculate growth medium, and grown to saturation in shaker flasks or a bioreactor

according to well known techniques. Plasmid DNA can be purified using standard bioseparation technologies such as solid phase anion-exchange resins supplied by QIAGEN, Inc. (Valencia, California). If required, supercoiled DNA can be isolated from the open circular and linear forms using gel electrophoresis or other methods.

5 Purified plasmid DNA can be prepared for injection using a variety of formulations. The simplest of these is reconstitution of lyophilized DNA in sterile phosphate-buffer saline (PBS). This approach, known as "naked DNA," is currently being used for intramuscular (IM) administration in clinical trials. To maximize the immunotherapeutic effects of minigene DNA vaccines, an alternative method for
10 formulating purified plasmid DNA may be desirable. A variety of methods have been described, and new techniques may become available. Cationic lipids can also be used in the formulation (see, *e.g.*, as described by WO 93/24640; Mannino & Gould-Fogerite, *BioTechniques* 6(7): 682 (1988); U.S. Pat No. 5,279,833; WO 91/06309; and Felgner, *et al.*, *Proc. Nat'l Acad. Sci. USA* 84:7413 (1987). In addition, glycolipids, fusogenic
15 liposomes, peptides and compounds referred to collectively as protective, interactive, non-condensing compounds (PINC) could also be complexed to purified plasmid DNA to influence variables such as stability, intramuscular dispersion, or trafficking to specific organs or cell types.

Target cell sensitization can be used as a functional assay for expression and HLA
20 class I presentation of minigene-encoded CTL epitopes. For example, the plasmid DNA is introduced into a mammalian cell line that is suitable as a target for standard CTL chromium release assays. The transfection method used will be dependent on the final formulation. Electroporation can be used for "naked" DNA, whereas cationic lipids allow direct *in vitro* transfection. A plasmid expressing green fluorescent protein (GFP) can be
25 co-transfected to allow enrichment of transfected cells using fluorescence activated cell sorting (FACS). These cells are then chromium-51 (^{51}Cr) labeled and used as target cells for epitope-specific CTL lines; cytolysis, detected by ^{51}Cr release, indicates both production of, and HLA presentation of, minigene-encoded CTL epitopes. Expression of HTL epitopes may be evaluated in an analogous manner using assays to assess HTL
30 activity.

In vivo immunogenicity is a second approach for functional testing of minigene DNA formulations. Transgenic mice expressing appropriate human HLA proteins are immunized with the DNA product. The dose and route of administration are formulation dependent (*e.g.*, IM for DNA in PBS, intraperitoneal (IP) for lipid-complexed DNA).

Twenty-one days after immunization, splenocytes are harvested and restimulated for 1 week in the presence of peptides encoding each epitope being tested. Thereafter, for CTL effector cells, assays are conducted for cytolysis of peptide-loaded, ⁵¹Cr-labeled target cells using standard techniques. Lysis of target cells that were sensitized by HLA loaded
5 with peptide epitopes, corresponding to minigene-encoded epitopes, demonstrates DNA vaccine function for *in vivo* induction of CTLs. Immunogenicity of HTL epitopes is evaluated in transgenic mice in an analogous manner.

Alternatively, the nucleic acids can be administered using ballistic delivery as described, for instance, in U.S. Patent No. 5,204,253. Using this technique, particles
10 comprised solely of DNA are administered. In a further alternative embodiment, DNA can be adhered to particles, such as gold particles.

IV.K.2. Combinations of CTL Peptides with Helper Peptides

Vaccine compositions comprising the peptides of the present invention, or analogs
15 thereof, which have immunostimulatory activity may be modified to provide desired attributes, such as improved serum half life, or to enhance immunogenicity.

For instance, the ability of the peptides to induce CTL activity can be enhanced by linking the peptide to a sequence which contains at least one epitope that is capable of inducing a T helper cell response. The use of T helper epitopes in conjunction with CTL
20 epitopes to enhance immunogenicity is illustrated, for example, in co-pending U.S.S.N. 08/820360, U.S.S.N. 08/197,484, and U.S.S.N. 08/464,234.

Particularly preferred CTL epitope/HTL epitope conjugates are linked by a spacer molecule. The spacer is typically comprised of relatively small, neutral molecules, such as amino acids or amino acid mimetics, which are substantially uncharged under
25 physiological conditions. The spacers are typically selected from, *e.g.*, Ala, Gly, or other neutral spacers of nonpolar amino acids or neutral polar amino acids. It will be understood that the optionally present spacer need not be comprised of the same residues and thus may be a hetero- or homo-oligomer. When present, the spacer will usually be at least one or two residues, more usually three to six residues. Alternatively, the CTL
30 peptide may be linked to the T helper peptide without a spacer.

Although the CTL peptide epitope can be linked directly to the T helper peptide epitope, often CTL epitope/HTL epitope conjugates are linked by a spacer molecule. The spacer is typically comprised of relatively small, neutral molecules, such as amino acids or amino acid mimetics, which are substantially uncharged under physiological

conditions. The spacers are typically selected from, *e.g.*, Ala, Gly, or other neutral spacers of nonpolar amino acids or neutral polar amino acids. It will be understood that the optionally present spacer need not be comprised of the same residues and thus may be a hetero- or homo-oligomer. When present, the spacer will usually be at least one or two
5 residues, more usually three to six residues. The CTL peptide epitope can be linked to the T helper peptide epitope either directly or via a spacer either at the amino or carboxy terminus of the CTL peptide. The amino terminus of either the immunogenic peptide or the T helper peptide may be acylated.

HTL peptide epitopes can also be modified to alter their biological properties. For
10 example, peptides comprising HTL epitopes can contain D-amino acids to increase their resistance to proteases and thus extend their serum half-life. Also, the epitope peptides of the invention can be conjugated to other molecules such as lipids, proteins or sugars, or any other synthetic compounds, to increase their biological activity. Specifically, the T helper peptide can be conjugated to one or more palmitic acid chains at either the amino
15 or carboxyl termini.

In certain embodiments, the T helper peptide is one that is recognized by T helper cells present in the majority of the population. This can be accomplished by selecting amino acid sequences that bind to many, most, or all of the HLA class II molecules. These are known as "loosely HLA-restricted" or "promiscuous" T helper sequences.
20 Examples of amino acid sequences that are promiscuous include sequences from antigens such as tetanus toxoid at positions 830-843 (QYIKANSKFIGITE), *Plasmodium falciparum* CS protein at positions 378-398 (DIEKKIAKMEKASSVFNVNS), and Streptococcus 18kD protein at positions 116 (GAVDSILGGVATYGAA). Other examples include peptides bearing a DR 1-4-7 supermotif, or either of the DR3 motifs.

25 Alternatively, it is possible to prepare synthetic peptides capable of stimulating T helper lymphocytes, in a loosely HLA-restricted fashion, using amino acid sequences not found in nature (*see, e.g.*, PCT publication WO 95/07707). These synthetic compounds called Pan-DR-binding epitopes (*e.g.*, PADRE™, Epimmune, Inc., San Diego, CA) are designed to most preferably bind most HLA-DR (human HLA class II) molecules. For
30 instance, a pan-DR-binding epitope peptide having the formula: aKXVWANTLKAAa, where "X" is either cyclohexylalanine, phenylalanine, or tyrosine, and a is either D-alanine or L-alanine, has been found to bind to most HLA-DR alleles, and to stimulate the response of T helper lymphocytes from most individuals, regardless of their HLA type.

An alternative of a pan-DR binding epitope comprises all "L" natural amino acids and can be provided in the form of nucleic acids that encode the epitope.

In some embodiments it may be desirable to include in the pharmaceutical compositions of the invention at least one component which primes cytotoxic T lymphocytes. Lipids have been identified as agents capable of priming CTL *in vivo* against viral antigens. For example, palmitic acid residues can be attached to the ϵ - and α -amino groups of a lysine residue and then linked, *e.g.*, via one or more linking residues such as Gly, Gly-Gly-, Ser, Ser-Ser, or the like, to an immunogenic peptide. The lipidated peptide can then be administered either directly in a micelle or particle, incorporated into a liposome, or emulsified in an adjuvant, *e.g.*, incomplete Freund's adjuvant. In a preferred embodiment, a particularly effective immunogenic comprises palmitic acid attached to ϵ - and α - amino groups of Lys, which is attached via linkage, *e.g.*, Ser-Ser, to the amino terminus of the immunogenic peptide.

As another example of lipid priming of CTL responses, *E. coli* lipoproteins, such as tripalmitoyl-S-glycerylcysteinylserine (P₃CSS) can be used to prime virus specific CTL when covalently attached to an appropriate peptide. (*See, e.g.*, Deres, *et al.*, *Nature* 342:561, 1989). Peptides of the invention can be coupled to P₃CSS, for example, and the lipopeptide administered to an individual to specifically prime a CTL response to the target antigen. Moreover, because the induction of neutralizing antibodies can also be primed with P₃CSS-conjugated epitopes, two such compositions can be combined to more effectively elicit both humoral and cell-mediated responses to infection.

As noted herein, additional amino acids can be added to the termini of a peptide to provide for ease of linking peptides one to another, for coupling to a carrier support or larger peptide, for modifying the physical or chemical properties of the peptide or oligopeptide, or the like. Amino acids such as tyrosine, cysteine, lysine, glutamic or aspartic acid, or the like, can be introduced at the C- or N-terminus of the peptide or oligopeptide, particularly class I peptides. However, it is to be noted that modification at the carboxyl terminus of a CTL epitope may, in some cases, alter binding characteristics of the peptide. In addition, the peptide or oligopeptide sequences can differ from the natural sequence by being modified by terminal-NH₂ acylation, *e.g.*, by alkanoyl (C₁-C₂₀) or thioglycolyl acetylation, terminal-carboxyl amidation, *e.g.*, ammonia, methylamine, *etc.* In some instances these modifications may provide sites for linking to a support or other molecule.

Vaccine Compositions Comprising Dendritic Cells Pulsed with CTL and/or HTL Peptides

An embodiment of a vaccine composition in accordance with the invention comprises *ex vivo* administration of a cocktail of epitope-bearing peptides to PBMC, or
5 isolated DC therefrom, from the patient's blood. A pharmaceutical to facilitate harvesting of DC can be used, such as GM-CSF/IL-4. After pulsing the DC with peptides and prior to reinfusion into patients, the DC are washed to remove unbound peptides. In this embodiment, a vaccine comprises peptide-pulsed DCs which present the pulsed peptide epitopes complexed with HLA molecules on their surfaces. The vaccine is then
10 administered to the patient.

IV.L. Administration of Vaccines for Therapeutic or Prophylactic Purposes

The peptides of the present invention and pharmaceutical and vaccine compositions of the invention are useful for administration to mammals, particularly
15 humans, to treat and/or prevent HCV infection. Vaccine compositions containing the peptides of the invention are administered to a patient infected with HCV or to an individual susceptible to, or otherwise at risk for, HCV infection to elicit an immune response against HCV antigens and thus enhance the patient's own immune response capabilities. In therapeutic applications, peptide and/or nucleic acid compositions are
20 administered to a patient in an amount sufficient to elicit an effective CTL and/or HTL response to the virus antigen and to cure or at least partially arrest or slow symptoms and/or complications. An amount adequate to accomplish this is defined as "therapeutically effective dose." Amounts effective for this use will depend on, *e.g.*, the particular composition administered, the manner of administration, the stage and severity
25 of the disease being treated, the weight and general state of health of the patient, and the judgment of the prescribing physician.

The vaccine compositions of the invention may also be used purely as prophylactic agents. Generally the dosage for an initial prophylactic immunization generally occurs in a unit dosage range where the lower value is about 1, 5, 50, 500, or
30 1000 µg and the higher value is about 10,000; 20,000; 30,000; or 50,000 µg. Dosage values for a human typically range from about 500 µg to about 50,000 µg per 70 kilogram patient. This is followed by boosting dosages of between about 1.0 µg to about 50,000 µg of peptide administered at defined intervals from about four weeks to six months after the

initial administration of vaccine. The immunogenicity of the vaccine may be assessed by measuring the specific activity of CTL and HTL obtained from a sample of the patient's blood.

As noted above, peptides comprising CTL and/or HTL epitopes of the invention
5 induce immune responses when presented by HLA molecules and contacted with a CTL
or HTL specific for an epitope comprised by the peptide. The manner in which the
peptide is contacted with the CTL or HTL is not critical to the invention. For instance,
the peptide can be contacted with the CTL or HTL either *in vivo* or *in vitro*. If the
contacting occurs *in vivo*, the peptide itself can be administered to the patient, or other
10 vehicles, *e.g.*, DNA vectors encoding one or more peptides, viral vectors encoding the
peptide(s), liposomes and the like, can be used, as described herein. When the peptide is
contacted *in vitro*, the vaccinating agent can comprise a population of cells, *e.g.*, peptide-
pulsed dendritic cells, or TAA-specific CTLs, which have been induced by pulsing
antigen-presenting cells *in vitro* with the peptide. Such a cell population is subsequently
15 administered to a patient in a therapeutically effective dose.

The peptides or DNA encoding them can be administered individually or as
fusions of one or more peptide sequences.

For pharmaceutical compositions, the immunogenic peptides of the invention, or
DNA encoding them, are generally administered to an individual already infected with
20 HCV. The peptides or DNA encoding them can be administered individually or as
fusions of one or more peptide sequences. Those in the incubation phase or the acute
phase of infection can be treated with the immunogenic peptides separately or in
conjunction with other treatments, as appropriate.

For therapeutic use, administration should generally begin at the first diagnosis of
25 HCV infection. This is followed by boosting doses until at least symptoms are
substantially abated and for a period thereafter. In chronic infection, loading doses
followed by boosting doses may be required.

Treatment of an infected individual with the compositions of the invention may
hasten resolution of the infection in acutely infected individuals. For those individuals
30 susceptible (or predisposed) to developing chronic infection, the compositions are
particularly useful in methods for preventing the evolution from acute to chronic
infection. Where susceptible individuals are identified prior to or during infection, the
composition can be targeted to them, thus minimizing the need for administration to a
larger population.

The peptide or other compositions used for the treatment or prophylaxis of HCV infection can be used, *e.g.*, in persons who have not manifested symptoms of disease but who act as a disease vector. In this context, it is generally important to provide an amount of the peptide epitope delivered by a mode of administration sufficient to
5 effectively stimulate a cytotoxic T cell response; compositions which stimulate helper T cell responses can also be given in accordance with this embodiment of the invention.

The dosage for an initial therapeutic immunization generally occurs in a unit dosage range where the lower value is about 1, 5, 50, 500, or 1000 μg and the higher value is about 10,000; 20,000; 30,000; or 50,000 μg . Dosage values for a human
10 typically range from about 500 μg to about 50,000 μg per 70 kilogram patient. Boosting dosages of between about 1.0 μg to about 50,000 μg of peptide pursuant to a boosting regimen over weeks to months may be administered depending upon the patient's response and condition as determined by measuring the specific activity of CTL and HTL obtained from the patient's blood. The peptides and compositions of the present
15 invention may be employed in serious disease states, that is, life-threatening or potentially life threatening situations. In such cases, as a result of the minimal amounts of extraneous substances and the relative nontoxic nature of the peptides in preferred compositions of the invention, it is possible and may be felt desirable by the treating physician to administer substantial excesses of these peptide compositions relative to
20 these stated dosage amounts.

Thus, for treatment of chronic infection, a representative dose is in the range disclosed above, namely where the lower value is about 1, 5, 50, 500, or 1000 μg and the higher value is about 10,000; 20,000; 30,000; or 50,000 μg , preferably from about 500 μg to about 50,000 μg per 70 kilogram patient. Initial doses followed by boosting doses at
25 established intervals, *e.g.*, from four weeks to six months, may be required, possibly for a prolonged period of time to effectively immunize an individual. In the case of chronic infection, administration should continue until at least clinical symptoms or laboratory tests indicate that the viral infection has been eliminated or substantially abated and for a period thereafter. The dosages, routes of administration, and dose schedules are adjusted
30 in accordance with methodologies known in the art.

The pharmaceutical compositions for therapeutic treatment are intended for parenteral, topical, oral, intrathecal, or local administration. Preferably, the pharmaceutical compositions are administered parentally, *e.g.*, intravenously,

subcutaneously, intradermally, or intramuscularly. Thus, the invention provides compositions for parenteral administration which comprise a solution of the immunogenic peptides dissolved or suspended in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers may be used, *e.g.*, water, buffered water, 0.8% saline, 0.3% glycine, hyaluronic acid and the like. These compositions may be sterilized by conventional, well known sterilization techniques, or may be sterile filtered. The resulting aqueous solutions may be packaged for use as is, or lyophilized, the lyophilized preparation being combined with a sterile solution prior to administration. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH-adjusting and buffering agents, tonicity adjusting agents, wetting agents, preservatives, and the like, for example, sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, sorbitan monolaurate, triethanolamine oleate, *etc.*

The concentration of peptides of the invention in the pharmaceutical formulations can vary widely, *i.e.*, from less than about 0.1%, usually at or at least about 2% to as much as 20% to 50% or more by weight, and will be selected primarily by fluid volumes, viscosities, *etc.*, in accordance with the particular mode of administration selected.

A human unit dose form of the peptide composition is typically included in a pharmaceutical composition that comprises a human unit dose of an acceptable carrier, preferably an aqueous carrier, and is administered in a volume of fluid that is known by those of skill in the art to be used for administration of such compositions to humans (*see, e.g., Remington's Pharmaceutical Sciences*, 17th Edition, A. Gennaro, Editor, Mack Publishing Co., Easton, Pennsylvania, 1985).

The peptides of the invention may also be administered via liposomes, which serve to target the peptides to a particular tissue, such as lymphoid tissue, or to target selectively to infected cells, as well as to increase the half-life of the peptide composition. Liposomes include emulsions, foams, micelles, insoluble monolayers, liquid crystals, phospholipid dispersions, lamellar layers and the like. In these preparations, the peptide to be delivered is incorporated as part of a liposome, alone or in conjunction with a molecule which binds to a receptor prevalent among lymphoid cells, such as monoclonal antibodies which bind to the CD45 antigen, or with other therapeutic or immunogenic compositions. Thus, liposomes either filled or decorated with a desired peptide of the invention can be directed to the site of lymphoid cells, where the liposomes then deliver the peptide compositions. Liposomes for use in accordance with the invention are formed

from standard vesicle-forming lipids, which generally include neutral and negatively charged phospholipids and a sterol, such as cholesterol. The selection of lipids is generally guided by consideration of, *e.g.*, liposome size, acid lability and stability of the liposomes in the blood stream. A variety of methods are available for preparing
5 liposomes, as described in, *e.g.*, Szoka, *et al.*, *Ann. Rev. Biophys. Bioeng.* 9:467 (1980), and U.S. Patent Nos. 4,235,871, 4,501,728, 4,837,028, and 5,019,369.

For targeting cells of the immune system, a ligand to be incorporated into the liposome can include, *e.g.*, antibodies or fragments thereof specific for cell surface determinants of the desired immune system cells. A liposome suspension containing a
10 peptide may be administered intravenously, locally, topically, *etc.* in a dose which varies according to, *inter alia*, the manner of administration, the peptide being delivered, and the stage of the disease being treated.

For solid compositions, conventional nontoxic solid carriers may be used which include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium
15 stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like. For oral administration, a pharmaceutically acceptable nontoxic composition is formed by incorporating any of the normally employed excipients, such as those carriers previously listed, and generally 10-95% of active ingredient, that is, one or more peptides of the invention, and more preferably at a concentration of 25%-75%.

20 For aerosol administration, the immunogenic peptides are preferably supplied in finely divided form along with a surfactant and propellant. Typical percentages of peptides are 0.01%-20% by weight, preferably 1%-10%. The surfactant must, of course, be nontoxic, and preferably soluble in the propellant. Representative of such agents are the esters or partial esters of fatty acids containing from 6 to 22 carbon atoms, such as
25 caproic, octanoic, lauric, palmitic, stearic, linoleic, linolenic, olesteric and oleic acids with an aliphatic polyhydric alcohol or its cyclic anhydride. Mixed esters, such as mixed or natural glycerides may be employed. The surfactant may constitute 0.1%-20% by weight of the composition, preferably 0.25-5%. The balance of the composition is ordinarily propellant. A carrier can also be included, as desired, as with, *e.g.*, lecithin for intranasal
30 delivery.

IV.M. Kits

The peptide and nucleic acid compositions of this invention can be provided in kit form together with instructions for vaccine administration. Typically the kit would

include desired peptide compositions in a container, preferably in unit dosage form and instructions for administration. An alternative kit would include a minigene construct with desired nucleic acids of the invention in a container, preferably in unit dosage form together with instructions for administration. Lymphokines such as IL-2 or IL-12 may
5 also be included in the kit. Other kit components that may also be desirable include, for example, a sterile syringe, booster dosages, and other desired excipients.

The invention will be described in greater detail by way of specific examples. The following examples are offered for illustrative purposes, and are not intended to limit the invention in any manner. Those of skill in the art will readily recognize a variety of
10 non-critical parameters that can be changed or modified to yield alternative embodiments in accordance with the invention.

V. EXAMPLES

As in many viral diseases, there is evidence that clearance of HCV is mediated by
15 CTL. In a study of primary HCV infection in six chimpanzees, four progressed to chronic infection (Cooper *et al.*, abstract, 19th US-Japan Hepatitis Joint Panel Meeting, January 27-29, 1998). It was found that these four animals showed either no CTL response or a very narrowly focused response during early infection. In contrast, in the remaining two animals that resolved the infection, a broad CTL response was observed
20 against multiple HCV proteins, some of which were conserved. Weiner *et al.* (*Proc. Natl. Acad. Sci. USA* 92:2755-2759, 1995) demonstrated that viral escape, in which the epitopes presented to PATR class I molecules mutated, was linked with a progression toward chronic infection. These data show a role for the CTL in directing the course of HCV disease, and in shaping the genetic composition of HCV species in the persistently
25 infected host.

In work in humans, Koziel and co-workers have established the presence of HCV-specific CTL in liver infiltrates from patients with chronic HCV infection (Koziel *et al.*, *J. Immunol.* 149:3339, 1992; and Koziel *et al.*, *J. Virol.* 67:7522, 1993), and have also identified a number of CTL epitopes recognized in the context of several different HLA
30 class I molecules. Other investigators have shown that HCV-specific CTL can be detected in the peripheral blood of patients with chronic hepatitis C (Cerny *et al.*, *J. Clin. Invest.* 95:521, 1995; Cerny *et al.*, *Curr. Topics in Micro. and Immunol* 189:169, 1994; Cerny *et al.*, Abst. 2nd International Meeting on Hepatitis C and Related Viruses; La Jolla, CA, 1994; Battegay *et al.*, Abst. 2nd International Meeting on Hepatitis C and Related

Viruses; La Jolla, CA, 1994; Shirai *et al.*, *J. Virol.* 68:3334, 1994; Shirai *et al.*, *J. Immunol.* 154:2733, 1995; Battegay *et al.*, *J. Virol.* 69:2462, 1995). In addition, escape variants have been demonstrated in patients chronically infected with HCV (Chang *et al.*, *J. Clin. Invest.* 100:2376-2385, 1997; Tsai *et al.*, *Gastroenterology* 115:954-966, 1998).

- 5 The magnitude of the CTL responses observed in HCV-infected patients is, in general, higher than those observed in the case of chronic hepatitis B infection, suggesting that there is less impairment of specific T cell immunity than with HBV infection. The magnitude of CTL responses in HCV patients is, however, lower than those observed in HBV infected individuals who successfully cleared HBV infection.
- 10 These results support the understanding that HCV infected patients are capable of responding to active immunotherapy, and suggest that potentiation and increasing of T cell responses to HCV may be of use in therapy and prevention of chronic HCV infection (Prince, A. M. *FEMS Micro. Rev.* 14:273, 1994).

- Several groups have analyzed the potential role of HCV-specific CTL responses in disease resistance and pathogenesis. In some studies no correlation was found between
- 15 CTL viremia and CTL precursor frequency for individual HCV epitopes (Rehermann *et al.*, *J. Clin. Invest.* 98:1432-1440, 1996; Wong *et al.*, *J. Immunol.* 160:1479-1488, 1998). In other studies, however, it was shown that a clear correlation existed between levels of HCV infection and CTL responses, provided that the global response against multiple
- 20 CTL epitopes was considered (Rehermann *et al.*, *J. Virol.* 70:7092-7102, 1996). These data represent a strong rationale for development of vaccine constructs capable of inducing vigorous CTL responses directed against a multiplicity of conserved HCV-derived epitopes.

- Koziel and colleagues have demonstrated the presence of HCV-specific CTLs, as well as T helper cell responses, in exposed but seronegative individuals (Koziel *et al.*, *J. Infect. Diseases* 176:859-866, 1997). In addition, HCV-specific CTLs have been detected in healthy, seronegative family members of chronically HCV-infected patents, indicating that a protective immunity is established in absence of a detectable infection (Bronowicki
- 25 *et al.*, *J. Infect. Dis.* 176:518-522, 1997; Scognamiglio *et al.*, in preparation).

- 30 Experimental evidence also indicates that HTL epitopes play an important role in immune reactivity and defenses against HCV infection (Missale *et al.*, *J. Clin. Invest.* 98:706-714, 1996). Diepolder *et al.* (in *Lancet* 346:1006, 1995) have shown that a region of the NS3 gene (NS3 1007-1534) is recognized by patients who clear acute HCV infection, but is not seen by patients who develop chronic infection. Subsequent studies

showed that this particular region contain a highly cross-reactive HTL epitope (NS3 1248-1261), which binds with good affinity to 10 of 13 DR molecules tested, and is highly conserved in 30/33 different HCV isolates considered (Diepolder *et al.*, *J. Virol.* 71:6011-6019, 1997). These data suggested that directing HTL responses to this type of epitope (rather than to less cross-reactive and/or highly variable ones) will be of therapeutic and prophylactic benefit and strongly argue for inclusion of this and other epitopes with similar characteristics in HCV vaccine constructs.

The following examples illustrate identification, selection, and use of immunogenic Class I and Class II peptide epitopes for inclusion in vaccine compositions.

Example 1: HLA Class I and Class II Binding Assays

The following example of peptide binding to HLA molecules demonstrates quantification of binding affinities of HLA class I and class II peptides. Binding assays can be performed with peptides that are either motif-bearing or not motif-bearing.

Epstein-Barr virus (EBV)-transformed homozygous cell lines, fibroblasts, CIR, or 721.22 transfectants were used as sources of HLA class I molecules. The specific cell lines routinely used for purification of MHC class I and class II molecules are listed in Table XXIV. Cell lysates were prepared and HLA molecules purified in accordance with disclosed protocols (Sidney *et al.*, *Current Protocols in Immunology* 18.3.1 (1998); Sidney, *et al.*, *J. Immunol.* 154:247 (1995); Sette, *et al.*, *Mol. Immunol.* 31:813 (1994)). HLA molecules were purified from lysates by affinity chromatography. The lysate was passed over a column of Sepharose CL-4B beads coupled to an appropriate antibody. The antibodies used for the extraction of HLA from cell lysates are listed in Table XXV. The anti-HLA column was then washed with 10mM Tris-HCL, pH 8.0, in 1% NP-40, PBS, and PBS containing 0.4% n-octylglucoside and HLA molecules were eluted with 50mM diethylamine in 0.15M NaCl containing 0.4% n-octylglucoside, pH 11.5. A 1/25 volume of 2.0M Tris, pH 6.8, was added to the eluate to reduce the pH to ~8.0. Eluates were then be concentrated by centrifugation in Centriprep 30 concentrators (Amicon, Beverly, MA). Protein content was evaluated by a BCA protein assay (Pierce Chemical Co., Rockford, IL) and confirmed by SDS-PAGE.

A detailed description of the protocol utilized to measure the binding of peptides to Class I and Class II MHC has been published (Sette *et al.*, *Mol. Immunol.* 31:813, 1994; Sidney *et al.*, in *Current Protocols in Immunology*, Margulies, Ed., John Wiley & Sons, New York, Section 18.3, 1998). Briefly, purified MHC molecules (5 to 500nM)

were incubated with various unlabeled peptide inhibitors and 1-10nM ^{125}I -radiolabeled probe peptides for 48h in PBS containing 0.05% Nonidet P-40 (NP40) (or 20% w/v digitonin for H-2 IA assays) in the presence of a protease inhibitor cocktail. All assays were at pH 7.0 with the exception of DRB1*0301, which was performed at pH 4.5, and

5 DRB1*1601 (DR2w21 β_1) and DRB4*0101 (DRw53), which were performed at pH 5.0.

Following incubation, MHC-peptide complexes were separated from free peptide by gel filtration on 7.8 mm x 15 cm TSK200 columns (TosoHaas 16215, Montgomeryville, PA). Because the large size of the radiolabeled peptide used for the DRB1*1501 (DR2w2 β_1) assay makes separation of bound from unbound peaks more

10 difficult under these conditions, all DRB1*1501 (DR2w2 β_1) assays were performed using a 7.8mm x 30cm TSK2000 column eluted at 0.6 mls/min. The eluate from the TSK columns was passed through a Beckman 170 radioisotope detector, and radioactivity was plotted and integrated using a Hewlett-Packard 3396A integrator, and the fraction of peptide bound was determined.

15 Radiolabeled peptides were iodinated using the chloramine-T method. Representative radiolabeled probe peptides utilized in each assay, and its assay specific IC_{50} nM, are summarized in Tables IV and V. Typically, in preliminary experiments, each MHC preparation was titrated in the presence of fixed amounts of radiolabeled peptides to determine the concentration of HLA molecules necessary to bind 10-20% of

20 the total radioactivity. All subsequent inhibition and direct binding assays were performed using these HLA concentrations.

Since under these conditions $[\text{label}] < [\text{HLA}]$ and $\text{IC}_{50} \geq [\text{HLA}]$, the measured IC_{50} values are reasonable approximations of the true K_D values. Peptide inhibitors are typically tested at concentrations ranging from 120 $\mu\text{g/ml}$ to 1.2 ng/ml, and are tested in

25 two to four completely independent experiments. To allow comparison of the data obtained in different experiments, a relative binding figure is calculated for each peptide by dividing the IC_{50} of a positive control for inhibition by the IC_{50} for each tested peptide (typically unlabeled versions of the radiolabeled probe peptide). For database purposes, and inter-experiment comparisons, relative binding values are compiled. These values

30 can subsequently be converted back into IC_{50} nM values by dividing the IC_{50} nM of the positive controls for inhibition by the relative binding of the peptide of interest. This method of data compilation has proven to be the most accurate and consistent for

comparing peptides that have been tested on different days, or with different lots of purified MHC.

Because the antibody used for HLA-DR purification (LB3.1) is α -chain specific, β_1 molecules are not separated from β_3 (and/or β_4 and β_5) molecules. The β_1 specificity of the binding assay is obvious in the cases of DRB1*0101 (DR1), DRB1*0802 (DR8w2), and DRB1*0803 (DR8w3), where no β_3 is expressed. It has also been demonstrated for DRB1*0301 (DR3) and DRB3*0101 (DR52a), DRB1*0401 (DR4w4), DRB1*0404 (DR4w14), DRB1*0405 (DR4w15), DRB1*1101 (DR5), DRB1*1201 (DR5w12), DRB1*1302 (DR6w19) and DRB1*0701 (DR7). The problem of β chain specificity for DRB1*1501 (DR2w2 β_1), DRB5*0101 (DR2w2 β_2), DRB1*1601 (DR2w21 β_1), DRB5*0201 (DR51Dw21), and DRB4*0101 (DRw53) assays is circumvented by the use of fibroblasts. Development and validation of assays with regard to DR β molecule specificity have been described previously (*see, e.g., Southwood et al., J. Immunol.* 160:3363-3373, 1998).

Binding assays as outlined above may be used to analyze supermotif and/or motif-bearing epitopes as, for example, described in Example 2.

Example 2. Identification of Conserved HLA Supermotif- and Motif-Bearing CTL Candidate Epitopes

Vaccine compositions of the invention may include multiple epitopes that comprise multiple HLA supermotifs or motifs to achieve broad population coverage. This example illustrates the identification of supermotif- and motif-bearing epitopes for the inclusion in such a vaccine composition. Calculation of population coverage was performed using the strategy described below.

Computer searches and algorithms for identification of supermotif and/or motif-bearing epitopes

Computer searches for epitopes bearing HLA Class I or Class II supermotifs or motifs were performed as follows. All translated HCV isolate sequences were analyzed using a text string search software program, *e.g.,* MotifSearch 1.4 (D. Brown, San Diego) to identify potential peptide sequences containing appropriate HLA binding motifs; alternative programs are readily produced in accordance with information in the art in view of the motif/supermotif disclosure herein. Furthermore, such calculations can be

made mentally. Identified A2-, A3-, and DR-supermotif sequences were scored using polynomial algorithms to predict their capacity to bind to specific HLA-Class I or Class II molecules. These polynomial algorithms take into account both extended and refined motifs (that is, to account for the impact of different amino acids at different positions),
5 and are essentially based on the premise that the overall affinity (or ΔG) of peptide-HLA molecule interactions can be approximated as a linear polynomial function of the type:

$$"\Delta G" = a_{1i} \times a_{2i} \times a_{3i} \dots \times a_{ni}$$

where a_{ji} is a coefficient which represents the effect of the presence of a given amino acid (j) at a given position (i) along the sequence of a peptide of n amino acids. The crucial
10 assumption of this method is that the effects at each position are essentially independent of each other (i.e., independent binding of individual side-chains). When residue j occurs at position i in the peptide, it is assumed to contribute a constant amount j_i to the free energy of binding of the peptide irrespective of the sequence of the rest of the peptide. This assumption is justified by studies from our laboratories that demonstrated that
15 peptides are bound to MHC and recognized by T cells in essentially an extended conformation (data omitted herein).

The method of derivation of specific algorithm coefficients has been described in Gulukota *et al.*, *J. Mol. Biol.* 267:1258-126, 1997; (see also Sidney *et al.*, *Human Immunol.* 45:79-93, 1996; and Southwood *et al.*, *J. Immunol.* 160:3363-3373, 1998).
20 Briefly, for all i positions, anchor and non-anchor alike, the geometric mean of the average relative binding (ARB) of all peptides carrying j is calculated relative to the remainder of the group, and used as the estimate of j_i . For Class II peptides, if multiple alignments are possible, only the highest scoring alignment is utilized, following an iterative procedure. To calculate an algorithm score of a given peptide in a test set, the
25 ARB values corresponding to the sequence of the peptide are multiplied. If this product exceeds a chosen threshold, the peptide is predicted to bind. Appropriate thresholds are chosen as a function of the degree of stringency of prediction desired.

Selection of HLA-A2 supertype cross-reactive peptides

30 Complete polypeptide sequences from fourteen HCV isolates were aligned, then scanned, utilizing motif identification software, to identify conserved 9- and 10-mer sequences containing the HLA-A2-supermotif main anchor specificity.

A total of 231 conserved, HLA-A2 supermotif-positive sequences were identified. These peptides were then evaluated for the presence of A*0201 preferred secondary anchor residues using A*0201-specific polynomial algorithms. A total of 67 conserved, motif-bearing and algorithm-positive sequences were identified.

5 Fifty of these conserved, motif-containing 9- and 10-mer peptides were tested for their capacity to bind to purified HLA-A*0201 molecules *in vitro* (HLA-A*0201 is considered a prototype A2 supertype molecule). Sixteen peptides bound A*0201 with IC_{50} values ≤ 500 nM; 4 with high binding affinities (IC_{50} values ≤ 50 nM) and 12 with intermediate binding affinities, in the 50-500 nM range (Table XXVI).

10 These 16 peptides were then tested for binding to additional A2-supertype molecules (A*0202, A*0203, A*0206, and A*6802). As shown in Table XXVI, most of these peptides were found to be A2-supertype cross-reactive binders. More specifically, 12/16 (75%) peptides bound at least three of the five A2-supertype molecules tested.

15 *Selection of HLA-A3 supermotif-bearing epitopes*

 The sequences from the same fourteen known HCV isolates scanned above were also examined for the presence of conserved peptides with the HLA-A3-supermotif primary anchors. A total of 71 conserved 9- or 10-mer motif containing sequences were identified. Further analysis using the A03 and A11 algorithms (see, e.g., Gulukota et al,
20 *J. Mol. Biol.* 267:1258-1267, 1997 and Sidney et al, *Human Immunol.* 45:79-93, 1996) identified 39 sequences that scored high in either or both algorithms. Twenty seven of the 39 peptides were synthesized and tested for binding to HLA-A*03 and HLA-A*11, the two most prevalent A3-supertype molecules. Fifteen peptides were identified which bound A3 and/or A11 with binding affinities of ≤ 500 nM (Table XXVII). These peptides
25 were then tested for binding cross-reactivity to the other common A3-supertype alleles (A*3101, A*3301, and A*6801). Seven of the 15 peptides bound at least three of the five HLA-A3-supertype molecules tested.

 In the course of an independent series of experiments (Kubo *et al.*, *J. Immunol.* 152:3913-3924, 1994), one peptide, HCV NS3 1262, not identified by the selection
30 criteria utilized above because it does not have the A3-supermotif main anchor specificity, was determined to be cross-reactive in the A3-supertype, binding A*03, A*11, and A*6801. It is also shown in Table XXVII. Interestingly, this peptide

represents a single residue N-terminal truncation of peptide 1073.14, which is also shown in Table XXVII.

In summary, 8 peptides that bind 3 or more A3-supertype molecules derived from conserved regions of the HCV genome were identified.

5

Selection of HLA-B7 supermotif bearing epitopes

When the same fourteen HCV isolates were also analyzed for the presence of conserved 9- or 10-mer peptides with the HLA-B7-supermotif, 35 sequences were identified. The corresponding peptides were synthesized and tested for binding to HLA-
10 B*0702, the most common B7-supertype allele (*i.e.*, the prototype B7 supertype allele). Thirteen peptides bound B*0702 with IC_{50} of ≤ 500 nM (Table XXVIIIa). These 13 peptides were then tested for binding to other common B7-supertype molecules (B*3501, B*51, B*5301, and B*5401). As shown in Table XXVIIIa, only 1 peptide (Core 169) was capable of binding to three or more of the five B7-supertype alleles tested.

15 To identify additional B7-supertype epitopes, further studies were undertaken. The protein sequences from the fourteen HCV isolates utilized above were again examined to identify conserved, motif-containing 8- and 11-mers. The isolates were also examined for 9- and 10-mer sequences allowing for lower conservancy (51%-78%). Twenty-five 8-mers, sixteen 11-mers, and thirty-five 9- and 10-mers were identified,
20 synthesized, and tested for binding to B*0702. Thirteen peptides bound with high or intermediate affinity ($IC_{50} \leq 500$ nM) (Table XXVIIIb). These peptides were additionally screened for binding to other B7-supertype molecules. Only one cross-reactive binder, the NS3 1378 8-mer (peptide 29.0035/1260.04), was identified (Table XXVIIIb).

In summary, a total of two cross-reactive B7-supertype binders were identified
25 (Core 169 and NS3 1378).

Selection of A1 and A24 motif-bearing epitopes

To further increase population coverage, HLA-A1 and -A24 epitopes can also be incorporated into potential vaccine constructs.

30 In a previous analysis, two A1 and three A24 binders, 100% conserved among four strains of HCV, were identified (Wentworth *et al.*, *Int. Immunol.* 8:651-659, 1996). An analysis of the protein sequence data from the fourteen HCV strains utilized above demonstrated that these peptides were >79% conserved, and also identified an additional

eleven A1- and twenty five A24-motif-containing conserved sequences (see Table XXIXA and B). Eight of the additional eleven A1 peptides and seven of the additional twenty five A24 peptides were tested for binding to the appropriate HLA molecule (*i.e.*, A1 or A24). Overall, as shown in Table XXIX, four A1-motif peptides (A) and three
5 A24-motif peptides (B) have been found with binding capacities of 500 nM or less for the appropriate allele-specific HLA molecule.

Analysis of the HLA-A2 and A3 supermotif-bearing epitopes identified above revealed that in 13/14 cases, peptides binding the supertype prototype HLA molecule (*i.e.* A*0201 for the A2 supertype, and A*0301 for the A3 supertype) with an IC₅₀ of less than
10 100nM were cross-reactive and recognized by HCV-infected patients as described in Example 3, which follows. Based on these observations, two A1 peptides and one A24 peptide epitopes were also selected as candidates for inclusion in vaccine compositions; these peptides bind the appropriate HLA molecule with an IC₅₀ of less than 100nM.

15 Example 3: Confirmation of Immunogenicity

*Evaluation of A*0201 immunogenicity*

It has been shown that CTL induced in A*0201/K^b transgenic mice exhibit specificity similar to CTL induced in the human system (*see, e.g.*, Vitiello *et al.*, *J. Exp. Med.* 173:1007-1015, 1991; Wentworth *et al.*, *Eur. J. Immunol.* 26:97-101, 1996).
20 Accordingly, these mice were used to evaluate the immunogenicity of the twelve conserved A2-supertype cross-reactive peptides identified in Example 2 above.

CTL induction in transgenic mice following peptide immunization has been described (Vitiello *et al.*, *J. Exp. Med.* 173:1007-1015, 1991; Alexander *et al.*; *J. Immunol.* 159:4753-4761, 1997). In these studies, mice were injected subcutaneously at
25 the base of the tail with each peptide (50 µg/mouse) emulsified in IFA in the presence of an excess of an IA^b-restricted helper peptide (140 µg/mouse) (HBV core 128-140, Sette *et al.*, *J. Immunol.* 153:5586-5592, 1994). Eleven days after injection, splenocytes were incubated in the presence of peptide-loaded syngenic LPS blasts. After six days, cultures were assayed for cytotoxic activity using peptide-pulsed targets. The data, summarized in
30 Table XXX, indicate that 7 of the 12 peptides (58%) were capable of inducing primary CTL responses in A*0201/K^b transgenic mice. (For these studies, a peptide was considered positive if it induced CTL (L.U. 30/10⁶ cells ≥2 in at least two transgenic animals (Wentworth *et al.*, *Eur. J. Immunol.* 26:97-101, 1996).

The conserved, cross reactive candidate CTL epitopes were also tested for recognition *in vitro* by PBMCs obtained from HCV-infected patients. Briefly, PBMC from patients infected with HCV were cultured in the presence of 10 µg/ml of synthetic peptide. After 7 and 14 days, the cultures were restimulated with peptide. The cultures
5 were assayed for cytolytic activity on day 21 using target cells pulsed with the specific peptide in a standard four hour ⁵¹Cr release assay. The data are summarized in Table XXX. As shown, all 12 peptides are CTL epitopes recognized by PBMC from HCV-infected patients. From the data in Table XXX, it is interesting to note that HLA transgenics did not fully reveal the immunogenicity of some peptides that were positive in
10 recall responses. This apparent discrepancy may reflect differences in the route of immunization utilized (*e.g.*, natural infection versus peptide immunization), or CTL repertoire.

*Evaluation of A*03/A11 immunogenicity*

15 The immunogenicity of six of the eight A3-supertype cross-reactive peptides identified in Example 2 above was evaluated in HLA-A11/K^b transgenic mice, using the protocol described above for HLA-A2 transgenic mice (Alexander *et al.*, *J. Immunol.* 159:4753-4761, 1997). Five of these six peptides were able to induce primary CTL responses (Table XXXI).

20 All eight peptides were also studied by collaborators using PBMC cultures from HCV infected patients and contacts of such patients. This data is also summarized in Table XXXI. Briefly, all eight peptides were recognized by HCV infected individuals.

Evaluation of B7 immunogenicity

25 One of the two B7-supertype cross-reactive peptides (1145.12, Core 169) has been evaluated for immunogenicity in HCV-infected patients. Two independent collaborators have shown that this peptide is indeed immunogenic, and is recognized by T cells from HCV-infected patients (Chang *et al.*, *J. Immunol.* 162:1156-1164, 1999)

30 Example 4: Implementation of the Extended Supermotif to Improve the Binding Capacity of Native Epitopes by Creating Analogs

HLA motifs and supermotifs (comprising primary and/or secondary residues) are useful in the identification and preparation of highly cross-reactive native peptides, as demonstrated herein. Moreover, the definition of HLA motifs and supermotifs also

allows one to engineer highly cross-reactive epitopes by identifying residues within a native peptide sequence which can be analogued, or "fixed" to confer upon the peptide certain characteristics, *e.g.* greater cross-reactivity within the group of HLA molecules that comprise a supertype, and/or greater binding affinity for some or all of those HLA molecules. Examples of analog peptides that exhibit modulated binding affinity are set forth in this example.

Analoging at Primary Anchor Residues

As shown in Example 2, more than ten different HCV-derived, A2-supertype-restricted epitopes were identified. Peptide engineering strategies are implemented to further increase the cross-reactivity of the candidate epitopes identified above which bind 3/5 of the A2 supertype alleles tested. On the basis of the data disclosed, *e.g.*, in related and co-pending U.S.S.N 09/226,775, the main anchors of A2-supermotif-bearing peptides are altered, for example, to introduce a preferred L, I, V, or M at position 2, and I or V at the C-terminus.

To analyze the cross-reactivity of the analog peptides, each engineered analog is initially tested for binding to the prototype A2 supertype allele A*0201, then, if A*0201 binding capacity is maintained, for A2-supertype cross-reactivity.

Similarly, analogs of HLA-A3 supermotif-bearing epitopes may also be generated. For example, peptides binding to 3/5 of the A3-supertype molecules may be engineered at primary anchor residues to possess a preferred residue (V, S, M, or A) at position 2.

The analog peptides are then tested for the ability to bind A*03 and A*11 (prototype A3 supertype alleles). Those peptides that demonstrate ≤ 500 nM binding capacity are then tested for A3-supertype cross-reactivity.

Similarly to the A2- and A3- motif bearing peptides, peptides binding 3 or more B7-supertype alleles may be improved, where possible, to achieve increased cross-reactive binding. B7 supermotif-bearing peptides may, for example, be engineered to possess a preferred residue (V, I, L, or F) at the C-terminal primary anchor position, as demonstrated by Sidney *et al.* (*J. Immunol.* 157:3480-3490, 1996).

Analoging at Secondary Anchor Residues

Moreover, HLA supermotifs are of value in engineering highly cross-reactive peptides and/or peptides that bind HLA molecules with increased affinity by identifying

particular residues at secondary anchor positions that are associated with such properties. Demonstrating this, the binding capacity of a peptide representing a discreet single amino acid substitution at position one was analyzed. Peptide 1145.13 (Table XXVIIIc), which represents the substitution of L to F at position 1 of the core 169 sequence, binds all five
5 B7-supertype molecules with a good affinity (all IC₅₀ values ≤ 132 nM), and in 3 instances has higher affinity over that of the parent peptide by >35-fold.

Because so few B7-supertype cross-reactive epitopes were identified, our results from previous binding evaluations were analyzed to identify conserved (8-, 9-, 10-, or 11-mer) peptides which bind, minimally, 3/5 B7 supertype molecules with weak affinity
10 (IC₅₀ of 500nM-5μM). This analysis identified 9 peptides, 6 of which are analogued (including core 169 which had been previously analogued). These peptides are tested for enhanced binding affinity and B7-supertype cross-reactivity.

Engineered analogs with sufficiently improved binding capacity or cross-reactivity are tested for immunogenicity in HLA-B7-transgenic mice, following for
15 example, IFA immunization or lipopeptide immunization.

In conclusion, these data demonstrate that by the use of even single amino acid substitutions, it is possible to increase the binding affinity and/or cross-reactivity of peptide ligands for HLA supertype molecules.

20 **Example 5: Identification of conserved HCV-derived sequences with HLA-DR binding motifs**

Peptide epitopes bearing an HLA class II supermotif or motif may also be identified as outlined below using methodology similar to that described in Examples 1-3.

25 ***Selection of HLA-DR-supermotif-bearing epitopes***

To identify HCV-derived, HLA class II HTL epitopes, the same fourteen HCV polyprotein sequences used for the identification of HLA Class I supermotif/motif sequences were analyzed for the presence of sequences bearing an HLA-DR-motif or supermotif. Specifically, 15-mer sequences were selected comprising a DR-supermotif,
30 further comprising a 9-mer core, and three-residue N- and C-terminal flanking regions (15 amino acids total). It was also required that the 15-mer sequence be conserved in at least 79% (11/14) of the HCV strains analyzed. These criteria identified a total of 49 non-redundant sequences, which are shown in Table XXXIIA. (In the context of Class II

epitopes, a sequence is considered operationally redundant if more than 80% of its sequence overlaps with another peptide.)

Protocols for predicting peptide binding to DR molecules have been developed (Southwood *et al.*, *J. Immunol.* 160:3363-3373, 1998). These protocols, specific for individual DR molecules, allow the scoring, and ranking, of 9-mer core regions. Each protocol not only scores peptide sequences for the presence of DR-supermotif primary anchors (i.e., at position 1 and position 6) within a 9-mer core, but additionally evaluates sequences for the presence of secondary anchors. Using allele specific selection tables (see, e.g., Southwood *et al.*, *ibid.*), it has been found that these protocols efficiently select peptide sequences with a high probability of binding a particular DR molecule. Additionally, it has been found that performing these protocols in tandem, specifically those for DR1, DR4w4, and DR7, can efficiently select DR cross-reactive peptides.

To see if these protocols serve to identify additional epitopes, the same HCV polyproteins used above were re-scanned for the presence of 15-mer peptides with 9-mer core regions that were $\geq 79\%$ (11/14 strains) conserved. This identified 152 sequences; 49 of which were identified previously, as described above. Next, the 9-mer core region of each of these peptides was scored using the DR1, DR4w4, and DR7 algorithms. Twenty-two peptides, including 12 new sequences (10 peptides were from the original set of 49) were found to have 9-mer cores with protocol-derived scores predictive of cross-reactive DR binders. The 12 additional sequences are shown in Table XXXIIB.

The conserved, HCV-derived peptides identified above were tested for their binding capacity for various common HLA-DR molecules. All peptides were initially tested for binding to the DR molecules in the primary panel: DR1, DR4w4, and DR7. Peptides binding at least 2 of these 3 DR molecules were then tested for binding to DR2w2 β 1, DR2w2 β 2, DR6w19, and DR9 molecules in secondary assays. Finally, peptides binding at least 2 of the 4 secondary panel DR molecules, and thus cumulatively at least 4 of 7 different DR molecules, were screened for binding to DR4w15, DR5w11, and DR8w2 molecules in tertiary assays. Peptides binding at least 7 of the 10 DR molecules comprising the primary, secondary, and tertiary screening assays were considered cross-reactive DR binders. The composition of these screening panels, and the phenotypic frequency of associated antigens, are shown in Table XXXIII.

Upon testing, it was found that 29 of the original 75 peptides (39%) bound two or more of the primary HLA molecules. Twenty-six of these cross-reactive binders were

then tested in the secondary assays, and nineteen were found to bind at least four of the seven HLA DR molecules in the primary and secondary panels. Finally, the nineteen peptides passing the secondary screening phase were tested for binding in the tertiary assays. As a result, nine peptides were identified which bound at least seven of ten
5 common HLA-DR molecules. Table XXXIV shows these nine peptides and their binding capacity for each allele-specific HLA-DR molecule in the primary through tertiary panels. Also shown in Table XXXIV are two peptides (F134.05 and F134.08) for which a complete binding analysis was not performed. However, both of these peptides bound six of the seven HLA DR molecules tested. F134.08 nests peptide 1283.44, which bound
10 eight of 10 allele-specific HLA molecules.

In conclusion, eleven cross-reactive DR-binding peptides, derived from six discrete (*i.e.* non-redundant) regions of the HCV genome, have been identified. Two of the six regions from which these epitopes were derived are covered by multiple, overlapping epitopes.

15

Selection of conserved DR3 motif peptides

Because HLA-DR3 is an allele that is prevalent in Caucasian, Black, and Hispanic populations, DR3 binding capacity is an important criterion in the selection of HTL epitopes. However, data generated previously indicated that DR3 only rarely cross-reacts
20 with other DR alleles (Sidney *et al.*, *J. Immunol.* 149:2634-2640, 1992; Geluk *et al.*, *J. Immunol.* 152:5742-5748, 1994; Southwood *et al.*, *J. Immunol.* 160:3363-3373, 1998). This is not entirely surprising in that the DR3 peptide-binding motif appears to be distinct from the specificity of most other DR alleles.

To efficiently identify peptides that bind DR3, target proteins were analyzed for
25 conserved sequences carrying one of the two DR3 specific binding motifs reported by Geluk *et al.* (*J. Immunol.* 152:5742-5748, 1994). Fifteen sequences, including a peptide nested within a DR-supermotif sequence identified above (peptide Pape 22), were identified (Table XXXIId). Preferably, DR3 motifs will be found clustered in proximity with DR supermotif regions.

30 Fourteen of the fifteen peptides containing a DR3 motif were tested for their DR3 binding capacity. Two peptides (CH35.0106 and CH35.0107) were found to bind DR3 with an affinity of 1 μ M or less (Table XXXV), and thereby qualify as HLA class II high affinity binders.

DR3 binding epitopes identified in this manner may then be included in vaccine compositions with DR supermotif-bearing peptide epitopes.

Example 6: Immunogenicity of candidate HCV-derived HTL epitopes and known dominant HCV HTL epitope

In the course of collaborative studies with G. Pape and C. Ferrari, eight conserved, HCV-derived peptides have been identified which are recognized by HCV-infected individuals.

One of these studies (Diepolder *et al.*, *J. Virol.* 71:6011-6019, 1997), identified peptide F98.05, which spans residues 1248-1261 of the NS3 protein, as an immunodominant CD4+ T-cell epitope that was recognized by 14/23 NS3-specific CD4+ T-cell clones from 4/5 patients with acute hepatitis C infection. This epitope, shown above to be an HLA-DR cross-reactive binder (see Table XXXIV), was capable of being presented to helper CD4+ T cells by multiple HLA molecules (DR4, DR11, DR12, DR13, and DR16). Two other peptides, Pape 22 and Pape 29, were also recognized by CD4+ T cell clones, although, in a more limited context; correspondingly, neither of these peptides are DR-cross-reactive binders.

By direct peripheral blood T cell stimulation and by fine specificity analysis of HCV-specific T-cell lines and clones, studies done in collaboration with Ferrari's group identified 6 immunodominant epitopes, including one also identified in the Pape collaboration, that are derived from conserved regions of the core, NS3, and NS4 proteins. These epitopes were also found to be cross-reactive, being presented to T cells in the context of different Class II molecules. Three of the 6 epitopes, F98.04 (F134.03), F134.05 and F134.08, are cross-reactive HLA-DR binders (see Table XXXIV).

In conclusion, the immunogenicity of 8 epitopes derived from conserved regions of the HCV genome has been demonstrated. Three of these epitopes (F98.05, F134.05, and F134.08; see Table XXXIV) are broadly cross-reactive HLA-DR binding peptides.

Example 7. Calculation of phenotypic frequencies of HLA-supertypes in various ethnic backgrounds to determine breadth of population coverage

This example illustrates the assessment of the breadth of population coverage of a vaccine composition comprised of multiple epitopes comprising multiple supermotifs and/or motifs.

In order to analyze population coverage, gene frequencies of HLA alleles were determined. Gene frequencies for each HLA allele were calculated from antigen or allele frequencies utilizing the binomial distribution formulae $gf=1-(\text{SQRT}(1-af))$ (see, *e.g.*, Sidney *et al.*, *Human Immunol.* 45:79-93, 1996). To obtain overall phenotypic

5 frequencies, cumulative gene frequencies were calculated, and the cumulative antigen frequencies derived by the use of the inverse formula $[af=1-(1-Cgf)^2]$.

Where frequency data was not available at the level of DNA typing, correspondence to the serologically defined antigen frequencies was assumed. To obtain total potential supertype population coverage no linkage disequilibrium was assumed, and

10 only alleles confirmed to belong to each of the superotypes were included (minimal estimates). Estimates of total potential coverage achieved by inter-loci combinations were made by adding to the A coverage the proportion of the non-A covered population that could be expected to be covered by the B alleles considered (*e.g.*, $\text{total}=A+B*(1-A)$). Confirmed members of the A3-like supertype are A3, A11, A31, A*3301, and A*6801.

15 Although the A3-like supertype may also include A34, A66, and A*7401, these alleles were not included in overall frequency calculations. Likewise, confirmed members of the A2-like supertype family are A*0201, A*0202, A*0203, A*0204, A*0205, A*0206, A*0207, A*6802, and A*6901. Finally, the B7-like supertype-confirmed alleles are: B7, B*3501-03, B51, B*5301, B*5401, B*5501-2, B*5601, B*6701, and B*7801 (potentially

20 also B*1401, B*3504-06, B*4201, and B*5602).

Population coverage achieved by combining the A2-, A3- and B7-superotypes is approximately 86% in five major ethnic groups (see Table XXI). Coverage may be extended by including peptides bearing the A1 and A24 motifs. On average, A1 is present in 12% and A24 in 29% of the population across five different major ethnic

25 groups (Caucasian, North American Black, Chinese, Japanese, and Hispanic). Together, these alleles are represented with an average frequency of 39% in these same ethnic populations. The total coverage across the major ethnicities when A1 and A24 are combined with the coverage of the A2-, A3- and B7-supertype alleles is >95%. An analogous approach can be used to estimate population coverage achieved with

30 combinations of class II motif-bearing epitopes.

Summary of candidate HLA class I and class II epitopes

In summary, on the basis of the data presented in the above examples, 26 CTL candidate peptide epitopes derived from conserved regions of the HCV virus have been

identified (Table XXXVIa). These include twelve HLA-A2 supermotif-bearing epitopes, eight HLA-A3 supermotif-bearing epitopes, and one HLA-B7 supermotif-bearing epitope, each capable of binding to multiple A2-, A3-, or B7-supertype molecules, and immunogenic in HLA transgenic mice or antigenic for human PBL (with the exception of peptide 29.0035/1260.04). Additional epitopes not evaluated for immunogenicity are also included. They are an additional B7-supermotif-bearing epitope and two HLA-A1 and one HLA-A24 high-affinity binding peptides. A known HLA-A31 restricted epitope (VGIYLLPNR), which also binds HLA-A33, is also set out in Table XXXVIa and is useful in combination with other Class I or Class II epitopes.

With these 26 CTL epitopes (as disclosed herein and from the art), average population coverage, (*i.e.*, recognition of at least one HCV epitope), is predicted to be greater than 95% in each of five major ethnic populations. The potential redundancy of coverage afforded by 25 of these epitopes (the peptide 24.0086 was not included) was estimated using the game theory Monte Carlo simulation analysis, which is known in the art (see *e.g.*, Osborne, M.J. and Rubinstein, A. "A course in game theory" MIT Press, 1994). As shown in Figure 1, it is estimated that 90% of the individuals in a population comprised of the Caucasian, North American Black, Japanese, Chinese, and Hispanic ethnic groups would recognize 2 or more of the candidate epitopes described herein.

A list of HCV-derived HTL epitopes that would be preferred for use in the design of minigene constructs or other vaccine formulations is summarized in Table XXXVIb. As shown, 9 different peptide-binding regions have been identified which bind multiple HLA-DR molecules or bind HLA-DR3. (In the case of the NS4 1914-1935 region, the longer peptide, F134.08, recognized by patients, was chosen over the shorter peptide, 1283.44. The longer peptide essentially incorporates the shorter peptide, and also binds additional DR molecules that the shorter peptide does not bind.) Three of these peptides have been recognized as dominant epitopes in HCV infected patients.

It is estimated that each of 10 common DR molecules recognizing the DR supermotif, and DR3, are covered by a minimum of 2 epitopes. Correspondingly, the total estimated population coverage represented by this panel of epitopes is in excess of 91% in each of the 5 major ethnic populations (Table XXXVII).

Example 8: Recognition Of Generation Of Endogenous Processed Antigens After Priming

This example determines that CTL induced by native or analogued peptide epitopes identified and selected as described in Examples 1-6 recognize endogenously synthesized, *i.e.*, native antigens.

Effector cells isolated from transgenic mice that are immunized with peptide epitopes as in Example 3, for example HLA-A2 supermotif-bearing epitopes, are re-stimulated *in vitro* using peptide-coated stimulator cells. Six days later, effector cells are assayed for cytotoxicity and the cell lines that contain peptide-specific cytotoxic activity are further re-stimulated. An additional six days later, these cell lines are tested for cytotoxic activity on ⁵¹Cr labeled Jurkat-A2.1/K^b target cells in the absence or presence of peptide, and also tested on ⁵¹Cr labeled target cells bearing the endogenously synthesized antigen, *i.e.* cells that are stably transfected with HCV expression vectors.

The result will demonstrate that CTL lines obtained from animals primed with peptide epitope recognize endogenously synthesized HCV antigen. The choice of transgenic mouse model to be used for such an analysis depends upon the epitope(s) that is being evaluated. In addition to HLA-A*0201/K^b transgenic mice, several other transgenic mouse models including mice with human A11, which may also be used to evaluate A3 epitopes, and B7 alleles have been characterized and others (*e.g.*, transgenic mice for HLA-A1 and A24) are being developed. HLA-DR1 and HLA-DR3 mouse models have also been developed, which may be used to evaluate HTL epitopes.

Example 9: Activity Of CTL-HTL Conjugated Epitopes In Transgenic Mice

This example illustrates the induction of CTLs and HTLs in transgenic mice by use of an HCV CTL/HTL peptide conjugate whereby the vaccine composition comprises peptides administered to an HCV-infected patient or an individual at risk for HCV. The peptide composition can comprise multiple CTL and/or HTL epitopes. This analysis demonstrates enhanced immunogenicity that can be achieved by inclusion of one or more HTL epitopes in a vaccine composition. Such a peptide composition can comprise a lipidated HTL epitope conjugated to a preferred CTL epitope containing, for example, at least one CTL epitope selected from Table XXVI-XXIX, or an analog of that epitope. The HTL epitope is, for example, selected from Table XXXII.

Lipopeptide preparation: Lipopeptides are prepared by coupling the appropriate fatty acid to the amino terminus of the resin bound peptide. A typical procedure is as

follows: A dichloromethane solution of a four-fold excess of a pre-formed symmetrical anhydride of the appropriate fatty acid is added to the resin and the mixture is allowed to react for two hours. The resin is washed with dichloromethane and dried. The resin is then treated with trifluoroacetic acid in the presence of appropriate scavengers [e.g. 5% (v/v) water] for 60 minutes at 20°C. After evaporation of excess trifluoroacetic acid, the crude peptide is washed with diethyl ether, dissolved in methanol and precipitated by the addition of water. The peptide is collected by filtration and dried.

Immunization procedures: Immunization of transgenic mice is performed as described (Alexander *et al.*, *J. Immunol.* 159:4753-4761, 1997). For example, A2/K^b mice, which are transgenic for the human HLA A2.1 allele and are useful for the assessment of the immunogenicity of HLA-A*0201 motif- or HLA-A2 supermotif-bearing epitopes, are primed subcutaneously (base of the tail) with 0.1 ml of peptide conjugate formulated in saline, or DMSO/saline. Seven days after priming, splenocytes obtained from these animals are restimulated with syngenic irradiated LPS-activated lymphoblasts coated with peptide.

Cell lines: Target cells for peptide-specific cytotoxicity assays are Jurkat cells transfected with the HLA-A2.1/K^b chimeric gene (*e.g.*, Vitiello *et al.*, *J. Exp. Med.* 173:1007, 1991)

In vitro CTL activation: One week after priming, spleen cells (30x10⁶ cells/flask) are co-cultured at 37°C with syngeneic, irradiated (3000 rads), peptide coated lymphoblasts (10x10⁶ cells/flask) in 10 ml of culture medium/T25 flask. After six days, effector cells are harvested and assayed for cytotoxic activity.

Assay for cytotoxic activity: Target cells (1.0 to 1.5x10⁶) are incubated at 37°C in the presence of 200 µl of ⁵¹Cr. After 60 minutes, cells are washed three times and resuspended in R10 medium. Peptide is added where required at a concentration of 1 µg/ml. For the assay, 10⁴ ⁵¹Cr-labeled target cells are added to different concentrations of effector cells (final volume of 200 µl) in U-bottom 96-well plates. After a 6 hour incubation period at 37°C, a 0.1 ml aliquot of supernatant is removed from each well and radioactivity is determined in a Micromedic automatic gamma counter. The percent specific lysis is determined by the formula: percent specific release = 100 x (experimental release - spontaneous release)/(maximum release - spontaneous release). To facilitate comparison between separate CTL assays run under the same conditions, % ⁵¹Cr release data is expressed as lytic units/10⁶ cells. One lytic unit is arbitrarily defined as the number of effector cells required to achieve 30% lysis of 10,000 target cells in a 6

hour ^{51}Cr release assay. To obtain specific lytic units/ 10^6 , the lytic units/ 10^6 obtained in the absence of peptide is subtracted from the lytic units/ 10^6 obtained in the presence of peptide. For example, if 30% ^{51}Cr release is obtained at the effector (E): target (T) ratio of 50:1 (i.e., 5×10^5 effector cells for 10,000 targets) in the absence of peptide and 5:1 (i.e., 5×10^4 effector cells for 10,000 targets) in the presence of peptide, the specific lytic units would be: $[(1/50,000) - (1/500,000)] \times 10^6 = 18 \text{ LU}$.

The results are analyzed to assess the magnitude of the CTL responses of animals injected with the immunogenic CTL/HTL conjugate vaccine preparation and are compared to the magnitude of the CTL response achieved using the CTL epitope as outlined in Example 3. Analyses similar to this may be performed to evaluate the immunogenicity of peptide conjugates containing multiple CTL epitopes and/or multiple HTL epitopes. In accordance with these procedures it is found that a CTL response is induced, and concomitantly that an HTL response is induced upon administration of such compositions.

15

Example 10. Selection of CTL and HTL epitopes for inclusion in an HCV-specific vaccine.

This example illustrates the procedure for the selection of peptide epitopes for vaccine compositions of the invention. The peptides in the composition can be in the form of a nucleic acid sequence, either single or one or more sequences (i.e., minigene) that encodes peptide(s), or may be single and/or polypeptidic peptides.

Epitopes are selected which, upon administration, mimic immune responses that have been observed to be correlated with tumor clearance. For example, vaccine can include 3-4 epitopes that come from at least one HCV antigen region. Epitopes from one region can be used in combination with epitopes from one or more additional HCV antigen regions. Analogs of epitopes can also be selected for inclusion in the vaccine.

Epitopes are often selected that have a binding affinity of an IC_{50} of 500 nM or less for an HLA class I molecule, or for class II, an IC_{50} of 1000 nM or less.

Sufficient supermotif bearing peptides, or a sufficient array of allele-specific motif bearing peptides, are selected to give broad population coverage. For example, epitopes are selected to provide at least 80% population coverage. A Monte Carlo analysis, a statistical evaluation known in the art, can be employed to assess breadth, or redundancy, of population coverage.

30

When creating a polyepitopic compositions, *e.g.* a minigene, it is typically desirable to generate the smallest peptide possible that encompasses the epitopes of interest. The principles employed are similar, if not the same, as those employed when selecting a peptide comprising nested epitopes. Additionally, however, upon
5 determination of the nucleic acid sequence to be provided as a minigene, the peptide sequence encoded thereby is analyzed to determine whether any "junctional epitopes" have been created. A junctional epitope is a potential HLA binding epitope, as predicted, *e.g.*, by motif analysis. Junctional epitopes are generally to be avoided because the recipient may bind to an HLA molecule and generate an immune response to that epitope,
10 which is not present in a native protein sequence.

Peptide epitopes for inclusion in vaccine compositions are, for example, selected from those listed in Tables XXVI-XXIX and Table XXXII. A vaccine composition comprised of selected peptides, when administered, is safe, efficacious, and elicits an immune response similar in magnitude of an immune response that clears an acute HCV
15 infection.

Example 11: Construction of Minigene Multi-Epitope DNA Plasmids

This example provides guidance for the construction of a minigene expression plasmid. Minigene plasmids may, of course, contain various configurations of CTL
20 and/or HTL epitopes or epitope analogs as described herein. Examples of the construction and evaluation of expression plasmids are described, for example, in co-pending U.S.S.N. 09/311,784 filed 5/13/99. An example of such a plasmid for the expression of HCV epitopes is shown in Figure 2, which illustrates the orientation of HCV peptide epitopes in a minigene construct.

25 A minigene expression plasmid may include multiple CTL and HTL peptide epitopes. In the present example, HLA-A2, -A3, -B7 supermotif-bearing peptide epitopes and HLA-A1 and -A24 motif-bearing peptide epitopes are used in conjunction with DR supermotif-bearing epitopes and/or DR3 epitopes (Figure 2). Preferred epitopes are identified, for example, in Tables XXVI-XXIX and XXXII. HLA class I supermotif or
30 motif-bearing peptide epitopes derived from multiple HCV antigens, *e.g.*, the core, NS4, NS3, NS5, NS1/E2, are selected such that multiple supermotifs/motifs are represented to ensure broad population coverage. Similarly, HLA class II epitopes are selected from multiple HCV antigens to provide broad population coverage, *i.e.* both HLA DR-1-4-7 supermotif-bearing epitopes and HLA DR-3 motif-bearing epitopes are selected for

inclusion in the minigene construct. The selected CTL and HTL epitopes are then incorporated into a minigene for expression in an expression vector.

This example illustrates the methods to be used for construction of such a minigene-bearing expression plasmid. Other expression vectors that may be used for
5 minigene compositions are available and known to those of skill in the art.

The minigene DNA plasmid contains a consensus Kozak sequence and a consensus murine kappa Ig-light chain signal sequence followed by CTL and/or HTL epitopes selected in accordance with principles disclosed herein. The sequence encodes an open reading frame fused to the Myc and His antibody epitope tag coded for by the
10 pcDNA 3.1 Myc-His vector.

Overlapping oligonucleotides, for example eight oligonucleotides, averaging approximately 70 nucleotides in length with 15 nucleotide overlaps, are synthesized and HPLC-purified. The oligonucleotides encode the selected peptide epitopes as well as appropriate linker nucleotides, Kozak sequence, and signal sequence. The final
15 multiepitope minigene is assembled by extending the overlapping oligonucleotides in three sets of reactions using PCR. A Perkin/Elmer 9600 PCR machine is used and a total of 30 cycles are performed using the following conditions: 95°C for 15 sec, annealing temperature (5° below the lowest calculated T_m of each primer pair) for 30 sec, and 72°C for 1 min.

For the first PCR reaction, 5 µg of each of two oligonucleotides, *i.e.*, an
20 amplification primer pair, are annealed and extended: Oligonucleotides 1+2, 3+4, 5+6, and 7+8 are combined in 100 µl reactions containing *Pfu* polymerase buffer (1x= 10 mM KCL, 10 mM (NH₄)₂SO₄, 20 mM Tris-chloride, pH 8.75, 2 mM MgSO₄, 0.1% Triton X-100, 100 µg/ml BSA), 0.25 mM each dNTP, and 2.5 U of *Pfu* polymerase. The full-
25 length dimer products are gel-purified, and two reactions containing the product of 1+2 and 3+4, and the product of 5+6 and 7+8 are mixed, annealed, and extended for 10 cycles. Half of the two reactions are then mixed, and 5 cycles of annealing and extension carried out before flanking primers are added to amplify the full length product for 25 additional cycles. The full-length product is gel-purified and cloned into pCR-blunt
30 (Invitrogen) and individual clones are screened by sequencing.

Example 12. The plasmid construct and the degree to which it induces immunogenicity.

The degree to which the plasmid construct prepared using the methodology outlined in Example 11 is able to induce immunogenicity is evaluated through *in vivo*

injections into mice and subsequent *in vitro* assessment of CTL and HTL activity, which are analysed using cytotoxicity and proliferation assays, respectively, as detailed *e.g.*, in U.S.S.N. 09/311,784 filed 5/13/99 and Alexander *et al.*, *Immunity* 1:751-761, 1994. For example, to assess the capacity of a pMin minigene construct that contains HLA-A2 supermotif epitopes to induce CTLs *in vivo*, HLA-A2.1/K^b transgenic mice are immunized intramuscularly with 100 µg of naked cDNA. As a means of comparing the level of CTLs induced by cDNA immunization, a control group of animals is also immunized with an actual peptide composition that comprises multiple epitopes synthesized as a single polypeptide as they would be encoded by the minigene.

Splenocytes from immunized animals are stimulated twice with each of the respective compositions (peptide epitopes encoded in the minigene or the polyepitopic peptide), then assayed for peptide-specific cytotoxic activity in a ⁵¹Cr release assay. The results indicate the magnitude of the CTL response directed against the A3-restricted epitope, thus indicating the *in vivo* immunogenicity of the minigene vaccine and polyepitopic vaccine. It is, therefore, found that the minigene elicits immune responses directed toward the HLA-A2 supermotif peptide epitopes as does the polyepitopic peptide vaccine. A similar analysis is also performed using other HLA-A3 and HLA-B7 transgenic mouse models to assess CTL induction by HLA-A3 and HLA-B7 motif or supermotif epitopes.

To assess the capacity of a class II epitope encoding minigene to induce HTLs *in vivo*, I-A^b restricted mice, for example, are immunized intramuscularly with 100 µg of plasmid DNA. As a means of comparing the level of HTLs induced by DNA immunization, a group of control animals is also immunized with an actual peptide composition emulsified in complete Freund's adjuvant.

CD4⁺ T cells, *i.e.* HTLs, are purified from splenocytes of immunized animals and stimulated with each of the respective compositions (peptides encoded in the minigene). The HTL response is measured using a ³H-thymidine incorporation proliferation assay, (*see, e.g.*, Alexander *et al.* *Immunity* 1:751-761, 1994). the results indicate the magnitude of the HTL response, thus demonstrating the *in vivo* immunogenicity of the minigene.

Alternatively, plasmid constructs can be evaluated *in vitro* by testing for epitope presentation by APC following transduction or transfection of the APC with an epitope-expressing nucleic acid construct. Such a study determines "antigenicity" and allows the use of human APC. The assay determines the ability of the epitope to be presented by the

APC in a context that is recognized by a T cell by quantifying the density of epitope-HLA class I complexes on the cell surface. Quantitation can be performed by directly measuring the amount of peptide eluted from the APC (*see, e.g., Sijts et al., J. Immunol.* 156:683-692, 1996; Demotz *et al., Nature* 342:682-684, 1989); or the number of peptide-HLA class I complexes can be estimated by measuring the amount of lysis or lymphokine release induced by infected or transfected target cells, and then determining the concentration of peptide necessary to obtained equivalent levels of lysis or lymphokine release (*see, e.g., Kageyama et al., J. Immunol.* 154:567-576, 1995).

10 Example 13: Peptide Composition for Prophylactic Uses

Vaccine compositions of the present invention are used to prevent HCV infection in persons who are at risk for such infection. For example, a polyepitopic peptide epitope composition (or a nucleic acid comprising the same) containing multiple CTL and HTL epitopes such as those selected in Examples 9 and/or 10, which are also selected to target greater than 80% of the population, is administered to individuals at risk for HCV infection. The composition is provided as a single lipidated polypeptide that encompasses multiple epitopes. The vaccine is administered in an aqueous carrier comprised of Freund's Incomplete Adjuvant. The dose of peptide for the initial immunization is from about 1 to about 50,000 µg, generally 100-5,000 µg, for a 70 kg patient. The initial administration of vaccine is followed by booster dosages at 4 weeks followed by evaluation of the magnitude of the immune response in the patient, by techniques that determine the presence of epitope-specific CTL populations in a PBMC sample. Additional booster doses are administered as required. The composition is found to be both safe and efficacious as a prophylaxis against HCV infection.

25 Alternatively, the polyepitopic peptide composition can be administered as a nucleic acid in accordance with methodologies known in the art and disclosed herein.

Example 14: Polyepitopic Vaccine Compositions Derived from Native HCV Sequences

A native HCV polyprotein sequence is screened, preferably using computer algorithms defined for each class I and/or class II supermotif or motif, to identify "relatively short" regions of the polyprotein that comprise multiple epitopes and is preferably less in length than an entire native antigen. This relatively short sequence that contains multiple distinct, even overlapping, epitopes is selected and used to generate a minigene construct. The construct is engineered to express the peptide, which

corresponds to the native protein sequence. The "relatively short" peptide is generally less than 250 amino acids in length, often less than 100 amino acids in length, preferably less than 75 amino acids in length, and more preferably less than 50 amino acids in length. The protein sequence of the vaccine composition is selected because it has
5 maximal number of epitopes contained within the sequence, *i.e.*, it has a high concentration of epitopes. As noted herein, epitope motifs may be nested or overlapping (*i.e.*, frame shifted relative to one another). For example, with frame shifted overlapping epitopes, two 9-mer epitopes and one 10-mer epitope can be present in a 10 amino acid peptide. Such a vaccine composition is administered for therapeutic or prophylactic
10 purposes.

The vaccine composition will preferably include, for example, three CTL epitopes and at least one HTL epitope from an HCV antigen. This polyepitopic native sequence is administered either as a peptide or as a nucleic acid sequence which encodes the peptide. Alternatively, an analog can be made of this native sequence, whereby one or more of the
15 epitopes comprise substitutions that alter the cross-reactivity and/or binding affinity properties of the polyepitopic peptide.

The embodiment of this example provides for the possibility that an as yet undiscovered aspect of immune system processing will apply to the native nested sequence and thereby facilitate the production of therapeutic or prophylactic immune
20 response-inducing vaccine compositions. Additionally such an embodiment provides for the possibility of motif-bearing epitopes for an HLA makeup that is presently unknown. Furthermore, this embodiment (absent analogs) directs the immune response to multiple peptide sequences that are actually present in native HCV antigens thus avoiding the need to evaluate any junctional epitopes. Lastly, the embodiment provides an economy of
25 scale when producing nucleic acid vaccine compositions.

Related to this embodiment, computer programs can be derived in accordance with principles in the art, which identify in a target sequence, the greatest number of epitopes per sequence length.

30 Example 15. Polyepitopic Vaccine Compositions Directed To Multiple Diseases

The HCV peptide epitopes of the present invention are used in conjunction with peptide epitopes from target antigens related to one or more other diseases, to create a vaccine composition that is useful for the prevention or treatment of HCV as well as the

one or more other disease(s). Examples of the other diseases include, but are not limited to, HIV, and HBV.

For example, a polyepitopic peptide composition comprising multiple CTL and HTL epitopes that target greater than 98% of the population may be created for administration to individuals at risk for both HCV and HIV infection. The composition can be provided as a single polypeptide that incorporates the multiple epitopes from the various disease-associated sources, or can be administered as a composition comprising one or more discrete epitopes.

10 Example 16. Use of peptides to evaluate an immune response

Peptides of the invention may be used to analyze an immune response for the presence of specific CTL or HTL populations directed to a prostate cancer-associated antigen. Such an analysis may be performed using multimeric complexes as described, *e.g.*, by Ogg *et al.*, *Science* 279:2103-2106, 1998 and Greten *et al.*, *Proc. Natl. Acad. Sci. USA* 95:7568-7573, 1998. In the following example, peptides in accordance with the invention are used as a reagent for diagnostic or prognostic purposes, not as an immunogen.

In this example, highly sensitive human leukocyte antigen tetrameric complexes ("tetramers") are used for a cross-sectional analysis of, for example, HCV HLA-A*0201-specific CTL frequencies from HLA A*0201-positive individuals at different stages of disease or following immunization using an HCV peptide containing an A*0201 motif. Tetrameric complexes are synthesized as described (Musey *et al.*, *N. Engl. J. Med.* 337:1267, 1997). Briefly, purified HLA heavy chain (A*0201 in this example) and β 2-microglobulin are synthesized by means of a prokaryotic expression system. The heavy chain is modified by deletion of the transmembrane-cytosolic tail and COOH-terminal addition of a sequence containing a BirA enzymatic biotinylation site. The heavy chain, β 2-microglobulin, and peptide are refolded by dilution. The 45-kD refolded product is isolated by fast protein liquid chromatography and then biotinylated by BirA in the presence of biotin (Sigma, St. Louis, Missouri), adenosine 5'triphosphate and magnesium. Streptavidin-phycoerythrin conjugate is added in a 1:4 molar ratio, and the tetrameric product is concentrated to 1 mg/ml. The resulting product is referred to as tetramer-phycoerythrin.

For the analysis of patient blood samples, approximately one million PBMCs are centrifuged at 300g for 5 minutes and resuspended in 50 μ l of cold phosphate-buffered saline. Tri-color analysis is performed with the tetramer-phycoerythrin, along with anti-CD8-Tricolor, and anti-CD38. The PBMCs are incubated with tetramer and antibodies on ice for 30 to 60 min and then washed twice before formaldehyde fixation. Gates are applied to contain >99.98% of control samples. Controls for the tetramers include both A*0201-negative individuals and A*0201-positive uninfected donors. The percentage of cells stained with the tetramer is then determined by flow cytometry. The results indicate the number of cells in the PBMC sample that contain epitope-restricted CTLs, thereby readily indicating the extent of immune response to the HCV epitope, and thus the stage of HCV infection or exposure to a vaccine that elicits a protective or therapeutic response.

Example 17: Use of Peptide Epitopes to Evaluate Recall Responses

The peptide epitopes of the invention are used as reagents to evaluate T cell responses, such as acute or recall responses, in patients. Such an analysis may be performed on patients who have recovered from infection, who are chronically infected with HCV, or who have been vaccinated with an HCV vaccine.

For example, the class I restricted CTL response of persons who have been vaccinated may be analyzed. The vaccine may be any HCV vaccine. PBMC are collected from vaccinated individuals and HLA typed. Appropriate peptide epitopes of the invention that are preferably highly conserved and, optimally, bear supermotifs to provide cross-reactivity with multiple HLA supertype family members, are then used for analysis of samples derived from individuals who bear that HLA type.

PBMC from vaccinated individuals are separated on Ficoll-Histopaque density gradients (Sigma Chemical Co., St. Louis, MO), washed three times in HBSS (GIBCO Laboratories), resuspended in RPMI-1640 (GIBCO Laboratories) supplemented with L-glutamine (2mM), penicillin (50U/ml), streptomycin (50 μ g/ml), and Hepes (10mM) containing 10% heat-inactivated human AB serum (complete RPMI) and plated using microculture formats. A synthetic peptide comprising an epitope of the invention is added at 10 μ g/ml to each well and HBV core 128-140 epitope is added at 1 μ g/ml to each well as a source of T cell help during the first week of stimulation.

In the microculture format, 4×10^5 PBMC are stimulated with peptide in 8 replicate cultures in 96-well round bottom plate in 100 μ l/well of complete RPMI. On

days 3 and 10, 100 ml of complete RPMI and 20 U/ml final concentration of rIL-2 are added to each well. On day 7 the cultures are transferred into a 96-well flat-bottom plate and restimulated with peptide, rIL-2 and 10^5 irradiated (3,000 rad) autologous feeder cells. The cultures are tested for cytotoxic activity on day 14. A positive CTL response
5 requires two or more of the eight replicate cultures to display greater than 10% specific ^{51}Cr release, based on comparison with uninfected control subjects as previously described (Rehermann, *et al.*, *Nature Med.* 2:1104,1108, 1996; Rehermann *et al.*, *J. Clin. Invest.* 97:1655-1665, 1996; and Rehermann *et al.* *J. Clin. Invest.* 98:1432-1440, 1996).

Target cell lines are autologous and allogeneic EBV-transformed B-LCL that are
10 either purchased from the American Society for Histocompatibility and Immunogenetics (ASHI, Boston, MA) or established from the pool of patients as described (Guilhot, *et al.* *J. Virol.* 66:2670-2678, 1992).

Cytotoxicity assays are performed in the following manner. Target cells consist of either allogeneic HLA-matched or autologous EBV-transformed B lymphoblastoid cell
15 line that are incubated overnight with the synthetic peptide epitope of the invention at 10 μM , and labeled with 100 μCi of ^{51}Cr (Amersham Corp., Arlington Heights, IL) for 1 hour after which they are washed four times with HBSS.

Cytolytic activity is determined in a standard 4-h, split well ^{51}Cr release assay using U-bottomed 96 well plates containing 3,000 targets/well. Stimulated PBMC are
20 tested at effector/target (E/T) ratios of 20-50:1 on day 14. Percent cytotoxicity is determined from the formula: $100 \times [(\text{experimental release} - \text{spontaneous release}) / (\text{maximum release} - \text{spontaneous release})]$. Maximum release is determined by lysis of targets by detergent (2% Triton X-100; Sigma Chemical Co., St. Louis, MO). Spontaneous release is <25% of maximum release for all experiments.

25 The results of such an analysis indicate the extent to which HLA-restricted CTL populations have been stimulated by previous exposure to HCV or an HCV vaccine.

The class II restricted HTL responses may also be analyzed. Purified PBMC are cultured in a 96-well flat bottom plate at a density of 1.5×10^5 cells/well and are stimulated with 10 $\mu\text{g}/\text{ml}$ synthetic peptide, whole antigen, or PHA. Cells are routinely plated in
30 replicates of 4-6 wells for each condition. After seven days of culture, the medium is removed and replaced with fresh medium containing 10U/ml IL-2. Two days later, 1 μCi ^3H -thymidine is added to each well and incubation is continued for an additional 18 hours. Cellular DNA is then harvested on glass fiber mats and analyzed for ^3H -thymidine

incorporation. Antigen-specific T cell proliferation is calculated as the ratio of ^3H -thymidine incorporation in the presence of antigen divided by the ^3H -thymidine incorporation in the absence of antigen.

5 **Example 18: Induction Of Specific CTL Response In Humans**

A human clinical trial for an immunogenic composition comprising CTL and HTL epitopes of the invention is set up as an IND Phase I, dose escalation study and carried out as a randomized, double-blind, placebo-controlled trial. Such a trial is designed, for example, as follows:

10 A total of about 27 subjects are enrolled and divided into 3 groups:

Group I: 3 subjects are injected with placebo and 6 subjects are injected with 5 μg of peptide composition;

Group II: 3 subjects are injected with placebo and 6 subjects are injected with 50 μg peptide composition;

15 Group III: 3 subjects are injected with placebo and 6 subjects are injected with 500 μg of peptide composition.

After 4 weeks following the first injection, all subjects receive a booster inoculation at the same dosage.

20 The endpoints measured in this study relate to the safety and tolerability of the peptide composition as well as its immunogenicity. Cellular immune responses to the peptide composition are an index of the intrinsic activity of this the peptide composition, and can therefore be viewed as a measure of biological efficacy. The following summarize the clinical and laboratory data that relate to safety and efficacy endpoints.

25 **Safety:** The incidence of adverse events is monitored in the placebo and drug treatment group and assessed in terms of degree and reversibility.

Evaluation of Vaccine Efficacy: For evaluation of vaccine efficacy, subjects are bled before and after injection. Peripheral blood mononuclear cells are isolated from fresh heparinized blood by Ficoll-Hypaque density gradient centrifugation, aliquoted in freezing media and stored frozen. Samples are assayed for CTL and HTL activity.

30 The vaccine is found to be both safe and efficacious.

Example 19: Phase II Trials In Patients Infected With HCV

Phase II trials are performed to study the effect of administering the CTL-HTL peptide compositions to patients having chronic HCV infection. The main objectives of

the trials are to determine an effective dose and regimen for inducing CTLs in chronically infected HCV patients, to establish the safety of inducing a CTL and HTL response in these patients, and to see to what extent activation of CTLs improves the clinical picture of chronically infected CTL patients, as manifested by a transient flare in alanine aminotransferase (ALT), normalization of ALT, and reduction in HCV DNA. Such a study is designed, for example, as follows:

The studies are performed in multiple centers. The trial design is an open-label, uncontrolled, dose escalation protocol wherein the peptide composition is administered as a single dose followed six weeks later by a single booster shot of the same dose. The dosages are 50, 500 and 5,000 micrograms per injection. Drug-associated adverse effects (severity and reversibility) are recorded.

There are three patient groupings. The first group is injected with 50 micrograms of the peptide composition and the second and third groups with 500 and 5,000 micrograms of peptide composition, respectively. The patients within each group range in age from 21-65, include both males and females, and represent diverse ethnic backgrounds. All of them are infected with HCV for over five years and are HIV, HBV and delta hepatitis virus (HDV) negative, but have positive levels of HCV antigen.

The magnitude and incidence of ALT flares and the levels of HCV DNA in the blood are monitored to assess the effects of administering the peptide compositions. The levels of HCV DNA in the blood are an indirect indication of the progress of treatment. The vaccine composition is found to be both safe and efficacious in the treatment of chronic HCV infection.

Example 20. Induction of CTL Responses Using a Prime Boost Protocol

A prime boost protocol can also be used for the administration of the vaccine to humans. Such a vaccine regimen may include an initial administration of, for example, naked DNA followed by a boost using recombinant virus encoding the vaccine, or recombinant protein/polypeptide or a peptide mixture administered in an adjuvant.

For example, the initial immunization may be performed using an expression vector, such as that constructed in Example 11, in the form of naked nucleic acid administered IM (or SC or ID) in the amounts of 0.5-5 mg at multiple sites. The nucleic acid (0.1 to 1000 µg) can also be administered using a gene gun. Following an incubation period of 3-4 weeks, a booster dose is administered. The booster can, *e.g.*, be recombinant fowlpox virus administered at a dose of $5 \cdot 10^7$ to $5 \cdot 10^9$ pfu. An alternative

recombinant virus, such as an MVA, canarypox, adenovirus, or adeno-associated virus, can also be used for the booster, or the polyepitopic protein or a mixture of the peptides can be administered. For evaluation of vaccine efficacy, patient blood samples will be obtained before immunization as well as at intervals following administration of the
5 initial vaccine and booster doses of the vaccine. Peripheral blood mononuclear cells are isolated from fresh heparinized blood by Ficoll-Hypaque density gradient centrifugation, aliquoted in freezing media and stored frozen. Samples are assayed for CTL and HTL activity.

Analysis of the results will indicate that a magnitude of response sufficient to
10 achieve protective immunity or to treat HCV infection is generated.

Example 21. Administration of Vaccine Compositions Using Dendritic Cells

Vaccines comprising peptide epitopes of the invention may be administered using dendritic cells. In this example, the peptide-pulsed dendritic cells can be administered to
15 a patient to stimulate a CTL response *in vivo*. In this method dendritic cells are isolated, expanded, and pulsed with a vaccine comprising peptide CTL and HTL epitopes of the invention. The dendritic cells are infused back into the patient to elicit CTL and HTL responses *in vivo*. The induced CTL and HTL then destroy (CTL) or facilitate destruction (HTL) of the specific target HCV-infected cells that bear the proteins from which the
20 epitopes in the vaccine are derived.

Alternatively, *Ex vivo* CTL or HTL responses to a particular tumor-associated antigen can be induced by incubating in tissue culture the patient's, or genetically compatible, CTL or HTL precursor cells together with a source of antigen-presenting cells, such as dendritic cells, and the appropriate immunogenic peptides. After an
25 appropriate incubation time (typically about 7-28 days), in which the precursor cells are activated and expanded into effector cells, the cells are infused back into the patient, where they will destroy (CTL) or facilitate destruction (HTL) of their specific target cells, *i.e.*, tumor cells.

Example 22: Alternative Method of Identifying Motif-Bearing Peptides

Another way of identifying motif-bearing peptides is to elute them from cells bearing defined MHC molecules. For example, EBV transformed B cell lines used for tissue typing, have been extensively characterized to determine which HLA molecules they express. In certain cases these cells express only a single type of HLA molecule.

These cells can then be infected with a pathogenic organism, *e.g.*, HCV, or transfected with nucleic acids that express the antigen of interest. Thereafter, peptides produced by endogenous antigen processing of peptides produced consequent to infection (or as a result of transfection) will bind be displayed on the cell surface. The peptides are then
5 eluted from the HLA molecules by exposure to mild acid conditions and their amino acid sequence determined, *e.g.*, by mass spectral analysis (*e.g.*, Kubo *et al.*, *J. Immunol.* 152:3913, 1994). Because, as disclosed herein, the majority of peptides that bind a particular HLA molecule are motif-bearing, this is an alternative modality for obtaining the motif-bearing peptides correlated with the particular HLA molecule expressed on the
10 cell.

Alternatively, cell lines that do not express any endogenous HLA molecules can be transfected with an expression construct encoding a single HLA allele. These cells may then be used as described, *i.e.*, they may be infected with a pathogenic organism or transfected with nucleic acid encoding an antigen of interest to isolate peptides
15 corresponding to the pathogen or antigen of interest that have been presented on the cell surface. Peptides obtained from such an analysis will bear motif(s) that correspond to binding to the single HLA allele that is expressed in the cell.

As appreciated by one in the art, one can perform a similar analysis on a cell bearing more than one HLA allele and subsequently determine peptides specific for each
20 HLA allele expressed. Moreover, one of skill would also recognize that means other than infection or transfection, such as loading with a protein antigen, can be used to provide a source of antigen to the cell.

The above examples are provided to illustrate the invention but not to limit its scope. For example, the human terminology for the Major Histocompatibility Complex, namely HLA, is used throughout this document. It is to be appreciated that these
25 principles can be extended to other species as well. Thus, other variants of the invention will be readily apparent to one of ordinary skill in the art and are encompassed by the appended claims. All publications, patents, and patent application cited herein are hereby incorporated by reference for all purposes.

TABLE I

SUPERMOTIFS	POSITION	POSITION	POSITION
	2 (Primary Anchor)	3 (Primary Anchor)	C Terminus (Primary Anchor)
A1	T , I , <i>L</i> , <i>V</i> , <i>M</i> , <i>S</i>		F , W , Y
A2	L , I , V , M , <i>A</i> , <i>T</i> , <i>Q</i>		I , V , <i>M</i> , <i>A</i> , <i>T</i> , <i>L</i>
A3	V , S , M , <i>A</i> , <i>T</i> , <i>L</i> , <i>I</i>		R , K
A24	Y , F , <i>W</i> , <i>I</i> , <i>V</i> , <i>L</i> , <i>M</i> , <i>T</i>		F , I , <i>Y</i> , <i>W</i> , <i>L</i> , <i>M</i>
B7	P		V , I , L , F , <i>M</i> , <i>W</i> , <i>Y</i> , <i>A</i>
B27	R , H , K		F , Y , L , <i>W</i> , <i>M</i> , <i>I</i> , <i>V</i> , <i>A</i>
B44	E , <i>D</i>		F , W , L , I , M , V , A
B58	A , T , S		F , W , Y , <i>L</i> , <i>I</i> , <i>V</i> , <i>M</i> , <i>A</i>
B62	Q , L , <i>I</i> , <i>V</i> , <i>M</i> , <i>P</i>		F , W , Y , <i>M</i> , <i>I</i> , <i>V</i> , <i>L</i> , <i>A</i>
MOTIFS			
A1	T , S , M		Y
A1		D , E , <i>A</i> , <i>S</i>	Y
A2.1	L , M , <i>V</i> , <i>Q</i> , <i>I</i> , <i>A</i> , <i>T</i>		V , <i>L</i> , <i>I</i> , <i>M</i> , <i>A</i> , <i>T</i>
A3	L , M , V , I , S , A , T , F , <i>C</i> , <i>G</i> , <i>D</i>		K , Y , R , <i>H</i> , <i>F</i> , <i>A</i>
A11	V , T , M , L , I , S , A , G , N , <i>C</i> , <i>D</i> , <i>F</i>		K , R , <i>Y</i> , <i>H</i>
A24	Y , F , W , <i>M</i>		F , L , I , W
A*3101	M , V , T , <i>A</i> , <i>L</i> , <i>I</i> , <i>S</i>		R , K
A*3301	M , V , A , L , F , <i>I</i> , <i>S</i> , <i>T</i>		R , K
A*6801	A , V , T , <i>M</i> , <i>S</i> , <i>L</i> , <i>I</i>		R , K
B*0702	P		L , M , F , <i>W</i> , <i>Y</i> , <i>A</i> , <i>I</i> , <i>V</i>
B*3501	P		L , M , F , W , Y , <i>I</i> , <i>V</i> , <i>A</i>
B51	P		L , I , V , F , <i>W</i> , <i>Y</i> , <i>A</i> , <i>M</i>
B*5301	P		I , M , F , W , Y , <i>A</i> , <i>L</i> , <i>V</i>
B*5401	P		A , T , I , V , <i>L</i> , <i>M</i> , <i>F</i> , <i>W</i> , <i>Y</i>

Bolded residues are preferred, italicized residues are less preferred: A peptide is considered motif-bearing if it has primary anchors at each primary anchor position for a motif or supermotif as specified in the above table.

TABLE II

SUPERMOTIFS	POSITION							
	1	2	3	4	5	6	7	8 C-terminus
A1		I° Anchor T,I,L,V,M,S						I° Anchor F,W,Y
A2		I° Anchor L,I,V,M,A, T,Q						I° Anchor L,I,V,M,A,T
A3	preferred	I° Anchor V,S,M,A,T, L,I	Y,F,W (4/5)		Y,F,W (3/5)	Y,F,W (4/5)	P (4/5)	I° Anchor R,K
	deleterious	D,E (3/5); P (5/5)	D,E (4/5)					
A24		I° Anchor Y,F,W,I,V, L,M,T						I° Anchor F,I,Y,W,L,M
B7	preferred	F,W,Y (5/5) L,I,V,M (3/5)	I° Anchor P	F,W,Y (4/5)			F,W,Y (3/5)	I° Anchor V,I,L,F,M,W,Y,A
	deleterious	D,E (3/5); P(5/5); G(4/5); A(3/5); Q,N (3/5)			D,E (3/5)	G (4/5)	Q,N (4/5)	D,E (4/5)
B27		I° Anchor R,H,K						I° Anchor F,Y,L,W,M,V,A
B44		I° Anchor E,D						I° Anchor F,W,Y,I,L,I,M,V,A
B58		I° Anchor A,T,S						I° Anchor F,W,Y,L,I,V,M,A
B62		I° Anchor Q,L,I,V,M, P						I° Anchor F,W,Y,M,I,V,L,A

POSITION

	1	2	3	4	5	6	7	8	C-terminus
<u>MOTIFS</u>									
A1 preferred 9-mer	G,F,Y,W	1°Anchor S,T,M	D,E,A	Y,F,W		P	D,E,Q,N	Y,F,W	1°Anchor Y
deleterious	D,E		R,H,K,L,I,V M,P	A	G	A			
A1 preferred 9-mer	G,R,H,K	A,S,T,C,L,I V,M,	1°Anchor D,E,A,S	G,S,T,C		A,S,T,C	L,I,V,M	D,E	1°Anchor Y
deleterious	A	R,H,K,D,E, P,Y,F,W		D,E	P,Q,N	R,H,K	P,G	G,P	

POSITION

	1	2	3	4	5	6	7	8	9 or C-terminus	C-terminus
A1 preferred 10-mer	Y,F,W	<u>1°Anchor</u> S,T,M	D,E,A,Q,N	A	Y,F,W,Q,N		P,A,S,T,C	G,D,E	P	<u>1°Anchor</u> Y
deleterious	G,P		R,H,K,G,L,I V,M	D,E	R,H,K	Q,N,A	R,H,K,Y,F, W	R,H,K	A	
A1 preferred 10-mer	Y,F,W	S,T,C,L,I,V M	<u>1°Anchor</u> D,E,A,S	A	Y,F,W		P,G	G	Y,F,W	<u>1°Anchor</u> Y
deleterious	R,H,K	R,H,K,D,E, P,Y,F,W			P	G		P,R,H,K	Q,N	
A2.1 preferred 9-mer	Y,F,W	<u>1°Anchor</u> L,M,I,V,Q, A,T	Y,F,W	S,T,C	Y,F,W		A	P	<u>1°Anchor</u> V,L,I,M,A,T	
deleterious	D,E,P		D,E,R,K,H			R,K,H	D,E,R,K,H			
A2.1 preferred 10-mer	A,Y,F,W	<u>1°Anchor</u> L,M,I,V,Q, A,T	L,V,I,M	G		G		F,Y,W, L,V,I,M		<u>1°Anchor</u> V,L,I,M,A,T
deleterious	D,E,P		D,E	R,K,H,A	P		R,K,H	D,E,R, K,H	R,K,H	

POSITION									
	1	2	3	4	5	6	7	8	9 or C-terminus
A3 preferred	R,H,K	1°Anchor L,M,V,I,S, A,T,F,C,G D	Y,F,W	P,R,H,K,Y, F,W	A	Y,F,W		P	C-terminus 1°Anchor K,Y,R,H,F,A
deleterious	D,E,P		D,E						
A11 preferred	A	1°Anchor V,T,L,M,I, S,A,G,N,C, D,F	Y,F,W	Y,F,W	A	Y,F,W	Y,F,W	P	1°Anchor K,R,Y,H
deleterious	D,E,P						A	G	
A24 9-mer preferred	Y,F,W,R,H,K	1°Anchor Y,F,W,M		S,T,C			Y,F,W	Y,F,W	1°Anchor F,I,I,W
deleterious	D,E,G		D,E	G	Q,N,P	D,E,R,H,K	G	A,Q,N	
A24 10-mer preferred		1°Anchor Y,F,W,M		P	Y,F,W,P		P		1°Anchor F,L,I,W
deleterious			G,D,E	Q,N	R,H,K	D,E	A	Q,N	D,E,A
A3101 preferred	R,H,K	1°Anchor M,V,T,A,L, I,S	Y,F,W	P		Y,F,W	Y,F,W	A,P	1°Anchor R,K
deleterious	D,E,P		D,E		A,D,E	D,E	D,E	D,E	

POSITION									
	1	2	3	4	5	6	7	8	9 or C-terminus C-terminus 1°Anchor R,K
A3301 preferred		1°Anchor M,V,A,L,F, I,S,T	Y,F,W				A,Y,F,W		
deleterious	G,P		D,E						
A6801 preferred	Y,F,W,S,T,C	1°Anchor A,V,T,M,S, L,I			Y,F,W,L,I, V,M		Y,F,W	P	1°Anchor R,K
deleterious	G,P		D,E,G		R,H,K			A	
B0702 preferred	R,H,K,F,W,Y	1°Anchor P	R,H,K		R,H,K	R,H,K	R,H,K	P,A	1°Anchor L,M,F,W,Y,A, I,V
deleterious	D,E,Q,N,P		D,E,P	D,E	D,E	G,D,E	Q,N	D,E	
B3501 preferred	F,W,Y,L,I,V,M	1°Anchor P	F,W,Y				F,W,Y		1°Anchor L,M,F,W,Y,I, V,A
deleterious	A,G,P				G	G			

POSITION									
	1	2	3	4	5	6	7	8	9 or C-terminus
B51	preferred L,I,V,M,F,W,Y	<i>1°Anchor</i> P	F,W,Y	S,T,C	F,W,Y		G	F,W,Y	C-terminus <i>1°Anchor</i> L,I,V,F,W, Y,A,M
	deleterious A,G,P,D,E,R,H,K, S,T,C				D,E	G	D,E,Q,N	G,D,E	
B5301	preferred L,I,V,M,F,W,Y	<i>1°Anchor</i> P	F,W,Y	S,T,C	F,W,Y		L,I,V,M,F, W,Y	F,W,Y	<i>1°Anchor</i> I,M,F,W,Y, A,L,V
	deleterious A,G,P,Q,N					G	R,H,K,Q,N	D,E	
B5401	preferred F,W,Y	<i>1°Anchor</i> P	F,W,Y,L,I,V M		L,I,V,M		A,I,L,I,V,M	F,W,Y,A,P	<i>1°Anchor</i> A,T,I,V,L, M,F,W,Y
	deleterious G,P,Q,N,D,E		G,D,E,S,T,C		R,H,K,D,E	D,E	Q,N,D,G,E	D,E	

Italicized residues indicate less preferred or "tolerated" residues.
The information in Table II is specific for 9-mers unless otherwise specified.

Table III

MOTIFS	POSITION					
	<u>1° anchor 1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>
DR4 preferred	F, M, Y, L, I, V, W	M	T		I	V, S, T, C, P, A, L, I, M
deleterious				W,		R, W, D, E
DR1 preferred	M, F, L, I, V, W, Y			P, A, M, Q		V, M, A, T, S, P, L, I, C
deleterious		C	C, H	F, D	C, W, D	G, D, E, D
DR7 preferred	M, F, L, I, V, W, Y	M	W	A		I, V, M, S, A, C, T, P, L
deleterious		C,		G,		G, R, D N
DR Supermotif	M, F, L, I, V, W, Y					V, M, S, T, A, C, P, L, I
DR3 MOTIFS	<u>1° anchor 1</u>	<u>2</u>	<u>3</u>	<u>1° anchor 4</u>	<u>5</u>	<u>1° anchor 6</u>
motif a preferred	L, I, V, M, F, Y			D		
motif b preferred	L, I, V, M, F, A, Y			D, N, Q, E, S, T		K, R, H

Italicized residues indicate less preferred or "tolerated" residues.

Table IV: HLA Class I Standard Peptide Binding Affinity.

ALLELE	STANDARD PEPTIDE	SEQUENCE (SEQ ID NO:)	STANDARD BINDING AFFINITY (nM)
A*0101	944.02	YLEPAIAKY	25
A*0201	941.01	FLPSDYFPSV	5.0
A*0202	941.01	FLPSDYFPSV	4.3
A*0203	941.01	FLPSDYFPSV	10
A*0205	941.01	FLPSDYFPSV	4.3
A*0206	941.01	FLPSDYFPSV	3.7
A*0207	941.01	FLPSDYFPSV	23
A*6802	1072.34	YVIKVSARV	8.0
A*0301	941.12	KVFPYALINK	11
A*1101	940.06	AVDLYHFLK	6.0
A*3101	941.12	KVFPYALINK	18
A*3301	1083.02	STLPETYVRR	29
A*6801	941.12	KVFPYALINK	8.0
A*2402	979.02	AYIDNYNKF	12
B*0702	1075.23	APRTLVL	5.5
B*3501	1021.05	FPFKYAAAF	7.2
B51	1021.05	FPFKYAAAF	5.5
B*5301	1021.05	FPFKYAAAF	9.3
B*5401	1021.05	FPFKYAAAF	10

Table V. HLA Class II Standard Peptide Binding Affinity.

Allele	Nomenclature	Standard Peptide	Sequence (SEQ ID NO:)	Binding Affinity (nM)
DRB1*0101	DR1	515.01	PKYVKQNTLKLAT	5.0
DRB1*0301	DR3	829.02	YKTIAFDEEARR	300
DRB1*0401	DR4w4	515.01	PKYVKQNTLKLAT	45
DRB1*0404	DR4w14	717.01	YARFQSQTTLKQKT	50
DRB1*0405	DR4w15	717.01	YARFQSQTTLKQKT	38
DRB1*0701	DR7	553.01	QYIKANSKFIGITE	25
DRB1*0802	DR8w2	553.01	QYIKANSKFIGITE	49
DRB1*0803	DR8w3	553.01	QYIKANSKFIGITE	1600
DRB1*0901	DR9	553.01	QYIKANSKFIGITE	75
DRB1*1101	DR5w11	553.01	QYIKANSKFIGITE	20
DRB1*1201	DR5w12	1200.05	EALIHQLKINPYVLS	298
DRB1*1302	DR6w19	650.22	QYIKANAKFIGITE	3.5
DRB1*1501	DR2w2 β 1	507.02	GRTQDENPVVHFFKNIV TPRTPPP	9.1
DRB3*0101	DR52a	511	NGQIGNDPNRDIL	470
DRB4*0101	DRw53	717.01	YARFQSQTTLKQKT	58
DRB5*0101	DR2w2 β 2	553.01	QYIKANSKFIGITE	20

Table VI

HLA-supertype	Allele-specific HLA-supertype members	
	Verified ^a	Predicted ^b
A1	A*0101, A*2501, A*2601, A*2602, A*3201	A*0102, A*2604, A*3601, A*4301, A*8001
A2	A*0201, A*0202, A*0203, A*0204, A*0205, A*0206, A*0207, A*0209, A*0214, A*6802, A*6901	A*0208, A*0210, A*0211, A*0212, A*0213
A3	A*0301, A*1101, A*3101, A*3301, A*6801	A*0302, A*1102, A*2603, A*3302, A*3303, A*3401, A*3402, A*6601, A*6602, A*7401
A24	A*2301, A*2402, A*3001	A*2403, A*2404, A*3002, A*3003
B7	B*0702, B*0703, B*0704, B*0705, B*1508, B*3501, B*3502, B*3503, B*3503, B*3504, B*3505, B*3506, B*3507, B*3508, B*5101, B*5102, B*5103, B*5104, B*5105, B*5301, B*5401, B*5501, B*5502, B*5601, B*5602, B*6701, B*7801	B*1511, B*4201, B*5901
B27	B*1401, B*1402, B*1509, B*2702, B*2703, B*2704, B*2705, B*2706, B*3801, B*3901, B*3902, B*7301	B*2701, B*2707, B*2708, B*3802, B*3903, B*3904, B*3905, B*4801, B*4802, B*1510, B*1518, B*1503
B44	B*1801, B*1802, B*3701, B*4402, B*4403, B*4404, B*4001, B*4002, B*4006	B*4101, B*4501, B*4701, B*4901, B*5001
B58	B*5701, B*5702, B*5801, B*5802, B*1516, B*1517	
B62	B*1501, B*1502, B*1513, B*5201	B*1301, B*1302, B*1504, B*1505, B*1506, B*1507, B*1515, B*1520, B*1521, B*1512, B*1514, B*1510

- Verified alleles include alleles whose specificity has been determined by pool sequencing analysis, peptide binding assays, or by analysis of the sequences of CTL epitopes.
- Predicted alleles are alleles whose specificity is predicted on the basis of B and F pocket structure to overlap with the supertype specificity.

Table VII
HCY A01 Super Motif with Binding Information

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)	A*0101
ATGNLPGCSF	165	10	13	93	
ATLGFAY	1285	8	14	100	
AVQWNRILAF	1917	11	14	100	
CTGSSQLY	1128	9	11	79	0.3700
CTRGVAKAVDF	1190	11	11	79	
CTWVNSTGF	555	9	11	79	
CVTQVDF	1482	8	12	88	
DLEWTSTW	1857	9	12	88	
ETTMRSVF	1207	9	12	88	
FSDTRCF	2870	8	11	79	
FTEAMTRY	2782	8	14	100	
FTGLTHIDAF	1567	11	13	93	
GLPVCDHLEF	1552	11	12	86	
GLSAFSLHSY	2821	10	11	79	0.0028
GLTHIDAF	1569	9	13	93	
GSSYGFQY	2841	8	11	79	
GTFFINAY	2063	8	11	78	
GVAGALVAF	1863	9	12	86	
GVAKAVDF	1193	8	11	79	
GVLAALAY	1670	9	12	86	
GVRCCEUALY	2819	11	14	100	
GVRLEQWNY	154	11	12	86	
HLICNVQVY	696	11	11	79	
HMVNFSGIY	1769	11	13	93	
HMGCEGVQW	1910	11	11	79	
INAKNEVF	2591	8	12	86	
ITYSTYGF	1296	8	12	86	
NDVQVLY	701	8	12	88	
KSTKVPAAV	1241	9	12	86	0.0130
KVDTLTQGF	121	10	12	86	
LEANLLW	2235	8	12	86	
LINTGWS	414	8	11	78	
LLAPITAY	1030	8	14	100	
LLNLGGW	1812	9	12	86	
LSPRGSRPSW	97	11	11	78	
LSAFSLHSY	2822	9	11	79	0.8100
LSPRGSRPSW	88	10	11	78	
LTCGFADLNGY	126	11	12	86	
LTHIDAF	1570	8	13	93	
LVDLAGY	1853	8	11	79	
MILMTHFF	2878	8	12	86	
NVDVQVLY	700	9	12	88	0.0980
NLPGCSF	168	10	13	93	
NTGVTVTVDF	1480	10	12	86	
NTNRRPDQNG	14	11	11	78	

HCY A01 Super Motif with Binding Information

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)	A*0101
NVQDLVGV	1108	8	11	79	
PITYSYGKF	1295	10	11	79	
PMGFSYOTRCF	2667	11	11	79	
PSVAATLGF	1281	9	14	100	
PTLHGPTLLY	1621	11	11	79	
PVOCHLEF	1554	9	12	86	
PVOCHLEFW	1554	10	12	86	
QTVDFSLDTF	1465	11	12	86	
RLHGLSAF	2918	8	12	88	
RLAPITAY	1020	9	12	86	
RMAYDMIMMW	317	10	12	86	
RMILMTHF	2875	8	12	86	
RMILMTIFF	2875	9	12	86	
RVCEKMAFY	2621	8	14	100	
RVEDGWNV	156	9	12	86	
STKVPAAY	1242	8	12	86	
SVAATLGF	1262	8	14	100	
SVAATLGFAY	1262	11	14	100	
TIMAKNEVF	2590	9	11	79	
TLHGPTRLY	1622	10	11	79	
TLFNLGGW	1811	10	12	86	
TTIMAKNEVF	2589	10	11	79	
TIIRSPVF	1208	8	12	86	
TVDFSLDTF	1466	10	12	86	
VIDLTCGF	122	9	12	86	
VLAALAY	1871	8	12	86	
VLEDGWNV	167	9	11	79	
VLDILAGY	1052	8	11	79	
VNGSYGCF	2639	8	11	79	
VNGSYGQY	2639	10	11	79	
WNNRLAF	1820	8	14	100	
YSPQRYEF	2848	9	11	79	
YTNVDLGVW	1106	11	11	79	
YVGLGGSVF	276	10	12	86	
		2			

0.0300

79

Table VIII
HCV A02 Super Motif with Binding Information

Conservancy	Freq.	Position	Sequence	A*0201	A*0202	A*0203	A*0206	A*8802
93	13	1904	ANLRHHV					
86	12	1873	ANLAAYCL					
79	11	1250	AAGYKVL					
79	11	1250	AAGYKVLV					
79	11	1250	AAGYKVLVL					
79	11	147	AARALAHGV					
79	11	147	AARALAHGVRV					
100	14	1284	AATLGFGA					
93	13	1284	AATLFGAYM					
86	12	1187	AAVCTRGV					
79	11	1187	AAVCTRGVA					
79	11	1187	AAVCTRGVAKA					
93	13	1890	AILSPGAL	0.0014				
86	12	1880	AILSPGALV	0.0035				
88	12	1890	AILSPGALW					
100	14	150	ALAHGVRV	0.0037				
100	14	150	ALAHGVRVL					
86	12	1737	ALGILDTA					
86	12	688	ALSTGLHL					
79	11	1896	ALVGVVCA					
79	11	1896	ALVGVVCAA					
79	11	1898	ALVGVVCAAI					
86	12	1602	ADAPPSWDQM	0.0160	0.0008	0.2200	0.0002	0.0039
79	11	1251	AQGYKVLV	0.0010				
79	11	1251	AQGYKVL					
86	12	77	AQPGYVPL					
93	13	1285	ATLFGAYM					
79	11	1354	ATPPGSVT					
79	11	1596	ATVCARADA					
100	14	1419	AVAYYRGL	0.0002				
100	14	1419	AVAYYRGLDV					
79	11	1188	AVCTRGVA					
79	11	1188	AVCTRGVAKA					
79	11	1188	AVCTRGVAKAV					
100	14	1917	AVQWNNRLI	0.0001				
100	14	1917	AVQWNNRLIA					
100	14	1903	CAALRRHV					
93	13	1530	CAWYELTPA					
86	12	2841	CLRLGVPL	0.0002				
86	12	738	CLWMLLI					
79	11	1653	CMSADLEV					

HCV A02 Super Motif with Binding Information

Conservancy	Freq.	Position	Sequence	A*0201	A*0202	A*0203	A*0206	A*6802
93	13	2673	DTRCFDSTV					
93	13	2673	DTRCFDSTV					
86	12	21	DVKTGGGQI	0.0001				
86	12	21	DVKTGGGQI					
79	11	750	EALLENLV					
100	14	2794	EAMTRYSA					
86	12	2237	EANLWROEM					
93	13	1377	EIPFYGKA	0.0001				
93	13	1377	EIPFYGKA	0.0002				
100	14	2814	ELTSCSSNW					
79	11	666	ELSPILLST					
79	11	666	ELSPILLST					
86	12	2245	EMGGINTRV	0.0003				
86	12	1731	EOROKAL					
86	12	1731	EOROKALGL					
86	12	1731	EOROKALGL					
86	12	1342	ETAGARLV					
86	12	1342	ETAGARLV					
86	12	1342	ETAGARLV					
86	12	1342	ETAGARLV					
86	12	1342	ETAGARLV					
86	12	1207	ETIMRSPV					
86	12	1207	ETIMRSPV					
86	12	1659	EWTSTWV	0.0001				
86	12	1659	EWTSTWV	0.0004				
86	12	1659	EWTSTWV					
93	13	130	FADLMGYI					
79	11	130	FADLMGYIPL					
79	11	130	FADLMGYIPL					
100	14	1927	FASRGNIV					
86	12	1927	FASRGNIVSPT					
100	14	1773	FISGHOYL	0.1000				
100	14	1773	FISGHOYL					
100	14	1773	FISGHOYL					
100	14	1773	FISGHOYL					
79	11	1304	FLADGGCSGA	0.0046				
86	12	177	FLALLSCL					
86	12	177	FLALLSCL					
93	13	728	FLALLSCLT					
86	12	1228	FOVAHLA	0.2800	0.0480	0.0670	0.0150	0.3500
86	12	1228	FOVAHLA					
79	11	2646	FOYSPGORV					
100	14	2792	FTEAMTRYSA					
93	13	1587	FTGLTHIDA					

ICV A02 Super Motif with Binding Information

Conservancy	Freq.	Position	Sequence	A'0201	A'0202	A'0203	A'0206	A'6802
93	13	512	FTPSPVV					
93	13	512	FTPSPVWG					
93	13	512	FTPSPVWGT					
79	11	684	FTLPALST					
79	11	684	FTLPALSTGL					
79	11	146	GARALAHGV					
86	12	992	GADTAACGDI					
86	12	992	GADTAMCGDII					
86	12	1861	GAGVAGAL					
86	12	1861	GAGVAGALV					
86	12	1861	GAGVAGALVA					
86	12	350	GAHMGVLA					
79	11	1895	GALWGW					
79	11	1895	GALWGWCA					
79	11	1895	GALWGWCAA					
86	12	1345	GARLVLA					
79	11	1345	GARLVLAT					
79	11	1345	GARLVLATATA					
79	11	1345	GARLVLATAT					
100	14	1816	GAVQWNRIL	0.0001				
100	14	1916	GAVQWNRIL					
100	14	1916	GAVQWNRILIA					
100	14	1333	GIGTLDQA					
100	14	1333	GIGTLDQAET					
100	14	1776	GIOYLAGL					
100	14	1776	GIOYLAGLST					
100	14	1776	GIOYLAGLSTL					
79	11	1425	GLDVSMT					
93	13	1552	GLPVODHL	0.0001				
79	11	968	GLROLAVA					
79	11	968	GLROLAVAV	0.0034				
100	14	1782	GLSTLPGNPA					
79	11	1782	GLSTLPGNPAI					
93	13	1569	GLTHIDAHFL					
93	13	28	GGTCCM	0.0007				
93	13	28	GGTCCM					
93	13	28	GGTCCM					
79	11	2063	GTFPINAYT					
79	11	2063	GTFPINAYTT					
100	14	1335	GTVDQNET					
100	14	1335	GTVDQNETA					
86	12	1863	GVAGALVA					
79	11	1081	GVQWVYHGA					

ILCV A02 Super Motif with Binding Information

Conservancy	Freq.	Position	Sequence	A*0201	A*0202	A*0203	A*0208	A*8802
86	12	1670	GVLAALAA					
86	12	1670	GVLAALAAAYCL					
79	11	161	GVNATIGNL	0.0001				
86	12	45	GVRAIRTKT					
100	14	2619	GVNCEKMA					
100	14	2619	GVNCEKMA					
100	14	2619	GVNCEKMA					
93	13	154	GVNLEDGV	0.0002				
79	11	1900	GVCAAIL	0.0001				
100	14	1234	IAPTGSGKST					
100	14	1572	HIDAFILSOT					
86	12	696	HJQINVDV	0.0100	0.0014	0.5400	0.0027	0.0037
79	11	1719	HJYIEOGM					
93	13	1769	HIMNFISGI					
79	11	698	IONNDVOYL	0.3300	0.0004	0.1300	0.0280	0.0053
79	11	222	HIPGCVPCV					
86	12	2855	HIPVNSWL					
86	12	2855	HIPVNSWLGN					
79	11	1910	HVGPGEA					
79	11	1910	HVGPGEAV					
86	12	1933	HVSPTHV					
100	14	1925	IAFASIGNHV					
79	11	1858	ILAGYGAGV	0.0430	0.0300	2.0000	0.0048	0.0450
79	11	1858	ILAGYGAGV	0.0002				
86	12	1816	ILGGWVA					
86	12	1816	ILGGWVAQL	0.0430	0.0024	0.0190	0.0005	0.0038
86	12	1816	ILGGWVAQLA					
86	12	1331	ILGIGTVL					
86	12	1331	ILGIGTVLDOA					
93	13	1891	ILSPGALV					
93	13	1891	ILSPGALV					
93	13	1891	ILSPGALV					
93	13	1891	ILSPGALVGV					
79	11	2591	IMAKNEVFCV	0.0210	0.0004	0.3700	0.0036	0.0130
100	14	1777	IOYLGLST	0.0088				
100	14	1777	IOYLGLSTL					
86	12	2250	ITRVESENKV					
86	12	2250	ITRVESENKV					
100	14	2816	ITSCSSNV					
100	14	2816	ITSCSSNVSV					
100	14	2816	ITSCSSNVSV					
86	12	909	ITWGADTA					
86	12	909	ITWGADTAA					

ILCV Δ02 Super Motif with Binding Information

Conservancy	Freq.	Position	Sequence	A*0201	A*0202	A*0203	A*0206	A*6802
79	11	1296	ITYSTYGMFL					
79	11	1296	ITYSTYGMFLA					
79	11	2813	NFPDLGV					
79	11	2813	NFPDLGVRV	0.0018				
93	13	30	WGGVYLL					
86	12	1738	KALGLLOT					
86	12	1738	KALGLLOTA					
86	12	2825	KMALYDVV					
86	12	1734	KOKALGIL					
86	12	1734	KOKVNLGLOT					
86	12	1734	KOKALGILLOTA					
88	12	121	KVIDTLTCGFA	0.0048				
100	14	1255	KVLVLPVS					
100	14	1255	KVLVLPVSVA					
100	14	1255	KVLVLPVSVA					
79	11	1244	KVPAAAYAA	0.0011				
86	12	1872	LAALAAAYCL					
79	11	1305	LADGGCSGGA					
86	12	1729	LAEOFKOKA					
86	12	1729	LAEOFKOKAL					
79	11	1857	LAGYGAGV					
79	11	1857	LAGYGAGVA					
79	11	1857	LAGYGAGVAGA					
100	14	151	LAHGVRL					
86	12	179	LALLSCLT					
79	11	972	LAVAVEPV					
100	14	1924	LIAFASRGNHV	0.0004				
100	14	2815	LITSCSSNV					
100	14	2815	LITSCSSNVSV	0.0002				
79	11	2612	LVFPDLGV					
79	11	2612	LVFPDLGVRV					
86	12	178	LLALLSCL					
86	12	178	LLALLSCLT	0.0230	0.0150	0.0220	0.0011	0.0130
100	14	728	LLFILLADA					
93	13	726	LLFILLADARV					
86	12	1812	LVFNILGGW	1.2000	0.0380	3.1000	0.1900	1.2000
86	12	1812	LVFNILGGWA					
93	13	729	LLADARV	0.0081				
93	13	1887	LLPAILSPGA					
93	13	1887	LLPAILSPGAL	0.0025				
83	13	38	LLPRRGPRIL					
83	13	36	LLPRRGPRILGV					

HCY A02 Super Motif with Binding Information

Conservancy	Freq.	Position	Sequence	A*0201	A*0202	A*0203	A*0206	A*8802
86	12	2240	LLWROEMGGN					
93	13	1629	LLYRLGAV					
79	11	133	LMGYRLV					
79	11	133	LMGYPLVGA					
86	12	2761	LOOCTMLV					
88	12	126	LTCBFADL					
88	12	126	LTCGFADLM					
100	14	2180	LTDPSSHIT					
100	14	2180	LTDPSHITA					
88	12	1052	LTGRDKQV					
93	13	1570	LTHIDAHFL					
83	13	2178	LTSMLTOPSHI					
79	11	2738	LTTCGNT					
79	11	2738	LTTCGNTL					
79	11	2738	LTTCGNTLT					
88	12	1591	LVAYQATV					
88	12	1591	LVAYQATVCA	0.0002				
79	11	1853	LVAILAGYGA	-0.0001				
86	12	1867	LVGGVLA	0.0003				
86	12	1667	LVGGVLAAL					
88	12	1667	LVGGVLAALA					
88	12	1667	LVGGVLAALAA					
100	14	1257	LVNPSVA					
100	14	1257	LVNPSVAA					
100	14	1257	LVNPSVAAT					
100	14	1257	LVNPSVAATL					
79	11	1884	LVNLLPAI					
79	11	1884	LVNLLPAIL	0.0002				
86	12	1137	LVTRHADV	0.0001				
79	11	1137	LVTRHADVI					
79	11	1137	LVTRHADVIPV					
79	11	1897	LVGVVCA					
79	11	1897	LVGVVCAA					
79	11	1897	LVGVVCAAI					
79	11	1897	LVGVVCAAIL	0.0011				
79	11	2773	LWICESA					
79	11	1348	LWLATAT					
86	12	2592	MMNEVFCV	0.0022				
86	12	2179	MLTDPSHI					
100	14	2179	MLTDPSHIT	0.0002				
100	14	2179	MLTDPSHITA					
83	13	322	MMMNWSPT					

HCY A02 Super Motif with Binding Information

Conservancy	Freq.	Position	Sequence	A*0201	A*0202	A*0203	A*0206	A*6802
93	13	1418	NAVAVYRGL					
93	13	1418	NAVAYYIGLDV					
88	12	2068	NAYTTGPCT					
86	12	1815	NILGGWVA					
86	12	1815	NILGSWAA					
88	12	1815	NILGGWAAQL					
93	13	1282	NIRTVRT	0.0001				
79	11	1282	NIRTVRTI					
79	11	1282	NIRTVRTIT					
79	11	1282	NIRTVRTITT					
86	12	2249	NITRVESENKV					
88	12	700	NIVDOYL					
88	12	118	NLGKVIDT	0.0006				
88	12	118	NLGKVIDTL					
86	12	118	NLGKVIDTLT					
93	13	1888	NLLPAILSPGA					
86	12	2239	NLIWROEM					
93	13	168	NLPGCSFSI	0.0041				
93	13	186	NLPGCSFSI					
86	12	1460	NTCVTQTV					
93	13	416	NTNGSMFI					
86	12	14	NINRPQDV					
93	13	1889	PAILSPGA					
93	13	1889	PAILSPGAL					
86	12	1889	PAILSPGALV					
88	12	1889	PAILSPGALVV					
86	12	688	PALSTGLI					
86	12	688	PALSTGLJHL					
79	11	2609	PARLVFPDL					
79	11	2066	PINAYTTGPCT					
79	11	1285	PITYSTYKFL					
93	13	2403	PLEGEPGDYDL					
79	11	143	PLGGAARA	0.0001				
79	11	143	PLGGAARAL					
79	11	143	PLGGAARALA					
93	13	1628	PLLYRLGA	0.0001				
93	13	1628	PLLYRLGAV					
79	11	2667	PMGFSYDT					
79	11	2807	POPEYDLEL					
79	11	2807	POPEYDLEU					
79	11	2807	POPEYDLEUT					
83	13	7	PORKTNT					

HCY A02 Super Modf with Binding Information

Conservancy	Freq.	Position	Sequence	A*0201	A*0202	A*0203	A*0206	A*6802
86	12	109	PTDPRRISRL					
79	11	1473	PTFTIETT					
79	11	1473	PTFTIETT					
100	14	1238	PTGSGKST					
93	13	1236	PTGSGKSTKV					
86	12	1935	PTHYPESDA					
86	12	1936	PTHYPESDAA					
79	11	1621	PTLHGPTPL					
78	11	1621	PTLHGPTPL					
78	11	2070	PTLWAFMI					
79	11	2870	PTLWARMIL					
79	11	2870	PTLWARMILM					
79	11	2870	PTLWARMILMT					
100	14	1628	PTPLLYRL					
93	13	1626	PTPLLYHLGA					
93	13	1626	PTPLLYRLGAV					
100	14	2857	PVNSWLGNI	0.0001				
100	14	2857	PVNSWLGNI	0.0001				
86	12	2857	PVNSWLGNIIM					
79	11	2318	PVNHGCP					
93	13	508	PVYCFPSPV	0.0004				
93	13	508	PVYCFPSPVV					
86	12	1340	QATAGARIL					
86	12	1340	QATAGARILV					
88	12	1340	QATAGARILV					
80	12	1803	QAPPSWDOM					
93	13	1595	QATVCAIYA					
79	11	1595	QATVCATQA					
93	13	29	QVGGVYL					
83	13	29	QVGGVYLL	0.0015				
88	12	338	QLLRPOA					
88	12	2184	QLPCEPEPV					
79	11	2210	QLSAPSLKA	0.0002				
79	11	2210	QLSAPSLKAT					
86	12	1455	QVCFGLCTT					
86	12	1229	QVAHLHAPT					
86	12	1186	RAAVCTRGV					
79	11	1186	RAAVCTRGVA					
100	14	149	RALAHGVV	0.0001				
100	14	149	RALAHGVVRL					
86	12	2733	RASGVLT					
79	11	43	RLGVRIATK					

HCV A02 Super Motif with Binding Information

Conservancy	Freq.	Position	Sequence	A*0201	A*0202	A*0203	A*0208	A*8802
78	11	2918	RHGLSAFSL					
79	11	2811	RLVFPDL	0.0280	0.0055	0.0180	0.0002	0.0032
79	11	2611	RLVFPDLGV					
79	11	1818	RLKPTLHGPT	0.0880	0.0110	1.0000	0.0100	0.0050
86	12	1029	RLAPITA					
86	12	1347	RLVLATA					
86	12	1347	RLVLATAT					
100	14	619	RLWHYPT					
86	12	317	RLWANDMM					
93	13	635	RLMYGGVEHL					
86	12	2243	RLDVGNI					
88	12	2243	RLDVGNI					
86	12	2243	RLDVGNI					
79	11	1284	RLDVGNI					
79	11	1284	RLDVGNI					
100	14	2521	RLDVGNI					
86	12	2521	RLDVGNI					
86	12	2522	RLDVGNI					
86	12	2522	RLDVGNI					
79	11	2100	RLDVGNI	0.0001				
86	12	156	RLDVGNI					
86	12	156	RLDVGNI					
86	12	2833	RLDVGNI					
79	11	1655	RLDVGNI					
79	11	1655	RLDVGNI					
79	11	2212	RLDVGNI					
79	11	2212	RLDVGNI					
93	13	2207	RLDVGNI					
100	14	175	RLDVGNI					
86	12	175	RLDVGNI					
100	14	1470	RLDVGNI					
86	12	1470	RLDVGNI					
79	11	1470	RLDVGNI					
79	11	2926	RLDVGNI					
86	12	1051	RLDVGNI					
100	14	2178	RLDVGNI					
100	14	2178	RLDVGNI					
100	14	2178	RLDVGNI					
86	12	2183	RLDVGNI					
86	12	2183	RLDVGNI					
93	13	2209	RLDVGNI					
79	11	2209	RLDVGNI					
78	11	2209	RLDVGNI					

LICV A02 Super Motif with Binding Information

Conservancy	Freq.	Position	Sequence	A*0201	A*0202	A*0203	A*0206	A*6802
93	13	56	SOPRGRROPI					
86	12	1242	STKVPAAYA					
79	11	1242	STKVPAAYAA					
100	14	1784	STLPGNPA					
79	11	1784	STLPGNPAI	0.0007				
79	11	2	SINPKPORT					
86	12	1663	STWLVGCV					
86	12	1663	STWLVGCVL					
86	12	1663	STWLVGCVLA					
88	12	1299	STYGKFLA					
100	14	1262	SVAATLGFCA					
86	12	1455	SVIDCNTCV					
86	12	1455	SVIDCNTCVT	0.0088				
88	12	995	TAAAGDII					
86	12	1343	TAGARLVV					
88	12	1343	TAGARLVWL					
88	12	1343	TAGARLVLA					
79	11	1343	TAGARLVVLA					
79	11	2852	TARHTPVNSWL					
79	11	2590	TIMAKNEV					
93	13	1268	TLGEGAYM					
88	12	1266	TLGEGAYMSKA					
79	11	1622	TLHGPTPL	0.0070				
79	11	1622	TLHGPTPLL					
86	12	1811	TLFNLGGW	0.0003				
79	11	686	TLPALSTGL	0.0004				
79	11	888	TLPALSTGLI					
78	11	1785	TLPGNPAI	0.0003				
86	12	125	TLTCGFADL					
88	12	125	TLTCGFADLM					
79	11	2871	TLWARMIL					
79	11	2871	TLWARMILM					
79	11	2871	TLWARMILMT					
88	12	1209	TMRSPVFT					
86	12	1404	TCTTDFEL					
86	12	1494	TOTVDFSLDPT					
79	11	2589	TTIMAKNEV					
79	11	685	TTLPALST					
79	11	685	TTLPALSTGL					
79	11	685	TTLPALSTGLI					
86	12	1208	TTMNSPVFT					
79	11	2739	TTSCGNIL					

HCY AD2 Super Motif with Binding Information

Conservancy	Freq.	Position	Sequence	A*0201	A*0202	A*0203	A*0208	A*6802
78	11	2739	TTSCGNLT					
78	11	1597	TVCARADA					
86	12	1466	TVDFSLDPT					
86	12	1466	TVDFSLDPTFT					
100	14	1336	TVDOAET					
100	14	1336	TVDOAETA					
88	12	1336	TVLDOAETAGA					
100	14	1263	VAATLGFBA					
93	13	1283	VAATLGFAYM					
86	12	1230	VAHLHAPT					
86	12	1440	VATDALMT					
86	12	1592	VAYQATVCA	0.0005				
79	11	1592	VAYQATVCARA	0.0001				
100	14	1420	VAYYRGLDV					
100	14	1420	VAYYRGLDVSV					
86	12	1456	VIDCNTCV					
86	12	1456	VIDCNTCVT					
88	12	1456	VIDCNTCVTOT					
88	12	122	VIDTLTGFA					
86	12	1671	VLAALAAAYCL	0.0500	0.0087	0.0047	0.0002	0.0550
93	13	1521	VLECECYDA					
79	11	1521	VLECECYDAGCA					
100	14	1337	VLDQAETA					
86	12	1337	VLDQAETAGA					
86	12	157	VLEDGVNYA					
88	12	157	VLEDGVNYAT					
100	14	1258	VLNPSVAA					
100	14	1258	VLNPSVAAT					
100	14	1258	VLNPSVAATL					
78	11	2737	VLTTSCGNT	0.0015				
78	11	2737	VLTTSCGNTL	0.0002				
79	11	2737	VLTTSCGNTLT					
79	11	1852	VLVDILAGYGA					
86	12	1666	VLVGGVLA					
88	12	1666	VLVGGVLAAL	0.0270	0.0130	0.3100	0.0120	0.0130
86	12	1666	VLVGGVLAALA	0.0084				
86	12	1666	VLVGGVLAALA					
100	14	1256	VLVLPNSV	0.0009				
100	14	1256	VLVLPNSVA					
100	14	1256	VLVLPNSVAA					
100	14	1256	VLVLPNSVAAT					
79	11	2600	VQFEGGRRDA					

HCY A02 Super Motif with Binding Information

Conservancy	Freq.	Position	Sequence	A*0201	A*0202	A*0203	A*0208	A*6802
79	11	1584	YOATVCARADA					
79	11	1106	YTNDDDL					
79	11	1106	YTNDDDLV					
86	12	276	YGDLCGSV	0.0018				
86	12	276	YGDLCGSVFL					
93	13	637	YGGVHFL	0.0008				
86	12	1939	YVPESDAA					
86	12	1938	YVPESDAAA					
86	12	1939	YVPESDAARV					
			555					

Table IX

ICV AD3 Super Motif (With Binding Information)

Conservancy	Freq.	Position	Sequence	A*0301	A*1101	A*3101	A*3301	A*8801
88	12	847	AACWTRGER					
79	11	147	AARALAHGVR					
79	11	1187	AAVCTRGVAK					
79	11	2208	ASOLSAPSLK					
86	12	1265	ATLGFAYMSK					
79	11	48	ATIKTSEI					
79	11	1188	AVCTRGVAK					
88	12	2941	CLVRLGVPTLR					
79	11	555	CTYMANSTGFTK					
79	11	2599	CYCFEKGGR					
79	11	2599	CYCFEKGGR					
100	14	1574	DAHFLSQTK					
93	13	2617	DLGVRVCEK					
79	11	1143	DVPIVPIR					
86	12	2245	EMGNITL					
86	12	2598	EYCFEKEK					
100	14	728	FLLLADAN					
79	11	148	GAARALAHGVR					
100	14	1918	GAVCMNIR					
79	11	3037	GYLLPNR					
79	11	1004	GLPVSARIR					
86	12	1131	GSSOLYLVR					
86	12	1883	GVAGALVAFK					
79	11	3035	GVGYLLPNR					
78	11	45	GVRATRTSER					
79	11	1800	GVVCAAILR					
79	11	1800	GVVCAAILR					
93	13	33	GYLLPNR					
93	13	33	GYLLPNR					
79	11	1141	HADVIPVR					
79	11	1141	HADVIPVR					
79	11	1141	HADVIPVR					
79	11	1141	HADVIPVR					
100	14	1234	HAPTSQSK					
93	13	1234	HAPTSQSK					
100	14	1572	HIDAHFLSQTK					
86	12	1232	HJHAPTSQSK					
100	14	1395	HLIFCHSK					
100	14	1395	HLIFCHSK					
100	14	1395	HLIFCHSK					
79	11	2828	HSYSNGEINR					
79	11	222	HITPGVPCVR					
86	12	2250	HTVESENK					
86	12	1298	ITYSTYCK					
79	11	2813	NTDLGVIR					
93	13	30	NGGVLLPR					
93	13	30	NGGVLLPR					
86	12	2844	KLGVPLLR					
86	12	10	KTKRNTNR					
86	12	10	KTKRNTNR					
93	13	51	KTSERSQPR					
86	12	51	KTSERSQPR					
86	12	1729	LADEKCK					

HCY A01 Shuer Motif (With Binding Information)

Conservancy	Freq	Position	Sequence	A'0301	A'1101	A'3101	A'3301	A'6801
86	12	2235	UEANLWR					
100	14	1398	LFCHSKK	0.0008	0.0005	0.0018	0.0088	0.0008
100	14	1298	LFCHSKK	0.5400	0.1800	0.0071	0.0012	0.0240
79	11	2612	UWFDLGV	0.0003	0.0001			
100	14	726	LLFLLADAR					
93	13	38	LIPTDPR					
86	12	87	LIPTDPR					
79	11	1591	LVAYQATVCAR					
79	11	1	MSINPKPQR					
79	11	1	MSINPKPQR					
86	12	2248	NTRVESENK	0.0010	0.0062			
79	11	14	NINRPODWK	0.0010	0.0007			
79	11	1295	PITYSTYQK					
79	11	2667	PMGFSYDTR					
83	13	514	PSVWVGITDR					
79	11	1807	PSMDMMWK					
86	12	108	PTDPTFTR	0.0008	0.0005			
83	13	1238	PTGSGKSTK	0.0002	0.0001	0.0008	0.0008	0.0002
93	13	518	PWVQITDR	0.0008	0.0005			
86	12	1340	QAEATAGAR					
93	13	28	QVGGVLLPR					
86	12	289	QLFTSPR	0.7500	0.0330	0.0290	0.0077	3.1000
79	11	289	QLFTSPR					
79	11	2210	QLSAPSUK					
79	11	1188	RAAVCTRGVAX					
100	14	149	RAAIGVR					
79	11	47	RATKTSER					
79	11	43	RLGVRAIR	0.8400	0.0280	0.0420	0.0004	0.0001
79	11	43	RLGVRAIR					
100	14	1823	RLNFAIR					
79	11	2611	RLVFDLGV					
100	14	635	RMVGVGR	0.7200	0.0200	0.1800	0.0030	0.0045
93	13	55	RSQRTGR					
79	11	2207	SASQLSAPSUK	0.0003	0.0044			
86	12	1132	SSDLVLTR					
79	11	2	STNPKPQR					
79	11	2	STNPKPQR					
79	11	2	STNPKPQR					
86	12	1268	TLGFOAYNSK	0.0810	0.0610	0.0005	0.0013	0.0009
79	11	1822	TUHGFTLLYR					
93	13	52	TSEPSQPR					
86	12	52	TSEPSQPR	0.0003	0.0001			
80	12	52	TSEPSQPR					
86	12	1050	TSIGDQK					
86	12	1884	VAGALYAFK					
79	11	1592	VAYQATVCAR					
86	12	1337	VLDQAEATAGAR	0.2400	0.8900	0.0048	0.0025	0.0310
86	12	1138	VITHADVPVR	0.0005	0.0038	0.0680	0.0720	0.0280
79	11	1901	VYCAAILR					
79	11	1901	VYCAAILR					
79	11	1898	VYGVCAAILR					
93	13	517	VWVGITDR					

HCV A01 Super Motif (With Binding Information)

Conservancy	Freq.	Position	Sequence	A*0301	A*1101	A*3101	A*3301	A*8801
86	12	93	WAGWLLSPR					
86	12	96	WLSPTQSR	0.0008	0.0005			
100	14	1920	WMNRLIAFASR					
79	11	557	WMNSTGFTK	0.0530	0.0810	0.0014	0.0420	0.0058
93	13	35	YLLPRGPR	0.0054	0.0005			
79	11	2930	YSPGENR					
100	14	637	YVGVEFR					
86	12	1939	YVPESDAAF	0.0003	0.0001			
			112					

Table X HCV A24 Super Motif With Binding Information

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)	A*2401
AILSPGAL	1890	8	13	93	
ALAHGVRVL	150	9	14	100	
ALSTGLIHL	689	9	12	86	
ALVGVVCAAI	1896	11	11	79	
ATGNLPGCSF	1896	10	13	93	
ATLGFAY	1265	6	14	100	
ATLGFAYM	1265	9	13	93	
AVAYYRGL	1419	8	14	100	
AVQWNNRL	1917	8	14	100	
AVQWNNRLI	1917	9	14	100	
AVQWNNRLIAF	1917	11	14	100	
AVQWNNMNV	319	8	12	86	
AVAAQGYKVL	1248	10	11	79	0.0009
AYYRGLDVSVI	1421	11	14	100	
CLRLKGVPL	2941	10	12	86	
CLWIMALLI	739	8	12	86	
CTGSSDL	1128	8	11	79	
CTGSSDLY	1128	9	11	79	
CTGSSDLYL	1128	10	11	79	
CTRGVAKAVDF	1190	11	11	79	
CTWNNSTGF	555	9	11	79	
CVTQTVDF	1462	8	12	86	
CVTQTVDFSL	1462	10	12	86	
CYDAGCAWY	1525	8	11	79	
CYDAGCAWY	1525	9	11	79	
CYDAGCAWYEL	1525	11	11	79	
DFSLDPTF	1488	8	14	100	
DFSLDPTFTI	1488	10	14	100	
DLCGSVRL	279	8	12	86	
DLEWTSW	1657	9	12	86	
DLEWTSWVL	1657	11	12	86	
DLGVRVCEKM	2617	10	13	93	
DLMGYIPL	132	8	11	79	
DLVNLPLAI	1883	9	11	79	
DLVNLPLAIL	1883	10	11	79	
DTAACGDI	994	8	12	86	
DTAACGDI	994	9	12	86	
DTLTCGFADL	124	10	12	86	
DTLTCGFADLM	124	11	12	86	
DWPFPGGQ	21	10	12	86	
DYPYRLWHY	615	9	14	100	
EIPFYKAI	1377	9	13	93	
ETAGARLWL	1342	10	12	86	
ETMRSPIV	1207	9	12	86	
EWTSTWVL	1659	9	12	86	

HICV A24 Super Motif With Binding Information

[illegible]

HCY A24 Super Motif With Binding Information

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)	A*2401
ILGIGTVL	1331	8	12	86	
IMANNEVF	2581	8	12	86	
ITYSTYGRF	1296	9	12	86	
ITYSTYCKRL	1296	10	11	79	
NOVOMLY	701	8	12	86	
NGGVYLL	30	8	13	93	
NFGGGOI	23	8	13	93	
KVIDLTGCF	121	10	12	86	
LFNIGGW	1813	8	12	86	
LEANLLW	2235	8	12	86	
LINTGSW	414	8	11	79	
LLALLSCL	170	8	12	86	
LLAPITAY	1030	8	14	00	
LLPRLOGW	1812	9	12	86	
LLPALLSPQAL	1887	11	13	93	
LLPRGGRLL	36	9	13	93	
LLSPRCSPSW	97	11	11	79	
LLWFOEMGGNI	2240	11	12	86	
LTCGFADL	128	8	12	86	
LTCGFADLM	128	9	12	86	
LTCGFADLMGY	128	11	12	86	
LTHDAHF	1570	8	13	93	
LTHDAHFL	1570	9	13	93	
LTSMLTDPSSH	2178	11	13	83	
LTISGNTL	2738	9	11	79	
LVDLAQY	1853	8	11	79	
LVGGVLAAL	1667	9	12	86	
LVLPNSVAATL	1257	11	14	100	
LVNLLPAI	1884	8	11	79	
LVNLLPAI	1884	8	11	79	
LVTRHADVI	1137	9	11	79	
LWGVWCAAI	1897	10	11	79	
LWGVWCAIL	1897	11	11	79	
LWARMILM	2872	8	12	86	
LWARMILMTHF	2872	11	12	86	
LWRCMGGN	2241	10	12	86	
LVVTRHADVI	1135	11	11	79	
MLMTIFF	2878	8	12	86	
MLTDPSH	2179	8	14	100	
MMNFISI	1770	8	14	100	
MMNFSQGY	1770	10	14	100	
MMNFSQGYL	1770	11	14	100	
MYGVGVEHFL	638	10	13	93	0.0270
NFSQGY	1772	8	14	100	
NFSQGYL	1772	9	14	100	0.0170

HCV A24 Super Motif With Binding Information

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)	A*2401
NLGGWAAQL	1815	11	12	86	
NRTGVRTI	1282	9	11	79	
NVDVQYL	700	8	12	86	
NVDVQYLY	700	9	12	86	0.0001
NLGVYDIL	118	9	12	86	
NLLWROEM	2239	8	12	86	
NLPQCSFSI	168	9	13	93	
NLPQCSFSF	168	10	13	93	
NLPQCSFSL	168	11	13	93	
NTCVDTQVDF	1460	10	12	86	
NTNGSMHI	416	8	13	93	
NTNRPQDVGF	14	11	11	79	
NWDDLGVW	1108	9	11	79	
NWFGCTWM	551	8	12	66	
PITYSTYGF	1295	10	11	79	
PITYSTYKFL	1295	11	11	79	
PLGEGGFPL	2403	11	13	79	
PLGGAARL	143	9	11	79	
PMGFSYDTRCF	2667	11	11	79	
PTDPRRSRL	109	11	12	86	
PTLHGPTPL	1621	9	11	78	
PTLHGPTPL	1821	10	11	79	
PTLHGPTPL	1621	11	11	79	
PTLWARM	2870	8	11	78	
PTLWARMIL	2870	9	11	79	
PTLWARMILM	2870	10	11	79	
PTLLYRL	1626	8	14	100	
PVODHIEF	1554	9	12	86	
PVODHLEFW	1554	10	12	86	
PVNSWLGNI	2857	9	14	100	
PVNSWLGNI	2857	10	14	100	
PVNSWLGNIH	2857	11	12	86	
PVHGCPL	2318	8	11	79	
QFKKALGL	1732	9	12	86	
QFKKALGL	1732	10	12	86	
QVGGVYL	29	8	13	93	
QVGGVYL	29	9	13	93	
QVDFSLDPTF	1465	11	12	86	
QVWNRUAF	1919	9	14	100	
QYLAGLSTL	1778	8	14	100	0.0480
QYSGORMEF	2647	10	11	79	0.0180
QYSGORVETL	2647	11	11	79	
RLHGLSAF	2918	8	12	86	
RLHGLSAFSL	2918	10	11	79	0.0001
RLVFPDL	2611	8	11	79	

HCV A24 Super Motif With Binding Information

Sequence	Position	Pepide No.	No. of Amino Acids	Sequence Frequency	Conservancy (%)	A*2401
RLAPITAY	1029		9	12	86	
RMAYDNMM	317		8	12	86	
RMAYDNMMANW	317		10	12	86	
RMILMTIF	2875		8	12	86	
RMILMTIFF	2875		9	12	86	
RMAYGVGDHFL	835		11	13	88	
RVCEKUAL	2621		8	14	93	
RVCEKUALY	2621		9	14	100	
RMLEDGAY	156		9	14	100	
SFSIFLLAL	173		9	14	86	
SFSIFLLAL	173		10	14	100	
SIFLLALL	175		8	14	100	
SIFLLALLSOL	175		11	12	100	0.0041
SLDPTFTI	1470		8	14	86	
SLHVSYPGEI	2928		10	11	100	
SMCTDPSH	2178		9	14	79	
STKVPAAJ	1242		8	12	100	
STLPGNPN	1784		9	11	86	
STWLVGGVL	1883		10	12	79	
SVAATLGF	1262		8	14	86	
SVAATLGFQAY	1508		11	14	100	
SWOQNMKOL	2860		9	11	100	
SWLGNIM	1164		8	12	79	
SYLKGSSGGFL	2590		11	12	88	
TIIMAKNEVF	1288		9	11	88	
TLOFGAYM	1622		8	13	79	
TLHGPTPL	1622		8	11	93	
TLHGPTPL	1622		9	11	79	
TUIGPTLLY	1622		10	11	79	
TLFNLGGW	1811		10	12	79	0.0001
TLPALSTGL	686		9	11	80	
TLPALSTGL	686		10	11	79	
TLPGNPAL	1785		8	11	79	
TLTGGFADL	125		9	11	79	
TLTGGFADLM	125		10	12	86	
TLWARMIL	2871		8	12	86	
TLWARMIL	2871		9	11	79	
TIIMAKNEVF	2588		9	11	79	
TIIPALSTGL	685		10	11	79	
TIIPALSTGL	685		10	11	79	
TIIPALSTGL	1208		11	12	79	
TIIPALSTGL	2139		8	12	86	
TIIPALSTGL	1468		8	12	79	
TIIPALSTGL	556		8	11	86	
TIIPALSTGL	1864		9	12	79	
TIIPALSTGL					86	

ICY A24 Super Motif With Binding Information

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)	A*2401
TYSTYKGF	1287	8	13	93	
TYSTYKRL	1297	9	12	86	
VFTGLTH	1586	8	13	93	0.0230
VIDTLTGF	122	9	12	86	
VLAALAY	1671	8	12	86	
VLAALAYCL	1671	10	12	86	0.0070
VLEGVNY	157	8	12	86	
VLPNSAATL	1258	10	14	100	
VLTSCGNTL	2737	10	11	79	
VLDILAGY	1852	9	11	79	
VLGGVLAAL	1868	10	12	86	
VMGSSYGF	2639	8	11	79	
VMGSSYGFQY	2639	10	11	79	
VTQVDFSL	1463	9	12	86	
VTRIADVI	1138	8	11	79	
WATDALM	1439	8	11	79	
WGWVCAAI	1898	9	11	79	
WGWVCAAIL	1898	10	11	79	
WTSIWYL	1860	8	12	86	
WYLPFRGPRL	34	11	13	83	0.0016
WYNRLAF	1920	8	14	100	
WVLVGGYL	1685	8	12	86	
WVLVGGVLAAL	1685	11	12	86	
YIPLVGAPL	136	9	11	79	
YLAGLSTL	1778	8	14	100	
YKSGSGRL	1165	10	12	86	
YKSGSGRLL	1165	11	12	86	
YLPTRIGPTL	35	10	13	93	0.0001
YLVTRHADVI	1136	10	11	79	
YTNDDCL	1108	8	11	79	
YTNDDCLVGH	1108	11	11	79	
YWGLOCSVF	276	10	12	86	
WGLQCSVFL	276	11	12	86	
WGGVEHFL	637	9	13	93	
YYRGLDVSVI	1422	10	14	100	
260		3			

Table XI

JCV B07 Super Motif (with Binding Information)

Conservancy	Freq.	Position	Sequence	B*0702	B*3501	B*5101	B*5301	B*5401
88	12	1604	APPSWDOM	0.0028	0.0002	0.0002	0.0001	0.0002
79	11	1604	APPSWDOMW	0.0001	0.0001	0.0002	0.0001	0.0002
93	13	1235	APTSCKSTKV	0.0001	0.0001	0.0002	0.0006	0.0003
79	11	2889	APTLWARM	0.4300	0.0001	0.0012	-0.0002	0.0023
79	11	2889	APTLWARM	0.0160	0.0002	0.0012	0.0001	0.0002
79	11	2889	APTLWARM	0.8000	0.0001	0.0010	0.0001	0.0003
79	11	2889	APTLWARMIL	0.0001	0.0001	-0.0003	-0.0002	0.0003
79	11	2889	APTLWARMILM	0.0130	0.0002	0.0002	0.0005	0.0002
79	11	2410	DFLSDQSW	0.0001	0.0002	0.0002	0.0001	0.0002
86	12	111	DFPRSNL	0.0170	0.0002	0.0001	0.0001	0.0002
79	11	2815	FRQGVV	0.0001	0.0001	0.0001	0.0001	0.0001
100	14	24	FRQGVV	0.0001	0.0001	0.0001	0.0001	0.0001
100	14	24	FRQGVV	0.0001	0.0001	0.0001	0.0001	0.0001
86	12	1912	FRQGVV	0.0001	0.0002	0.0002	0.0001	0.0002
86	12	1912	FRQGVV	0.0001	0.0001	0.0002	0.0001	0.0003
86	12	1912	FRQGVV	0.0001	0.0001	0.0002	0.0001	0.0002
93	13	41	GPTLVRA	0.0001	0.0002	0.0002	0.0001	0.0002
100	14	1825	GPTLVRL	0.0024	0.0002	0.0002	0.0001	0.0002
93	13	1625	GPTLVRLGA	0.0005	0.0001	0.0001	0.0001	0.0001
93	13	507	GPVYCFPSV	0.0001	0.0001	0.0001	0.0001	0.0001
93	13	1378	IPFYKAN	0.0120	0.0001	0.1200	-0.0002	0.2000
93	13	1378	IPFYKAN	0.4400	0.0032	0.0700	0.0003	0.0035
79	11	137	IPLVGAPL	0.0150	0.0002	0.0017	-0.0002	0.0006
86	12	2608	KPARLVF	0.0003	0.0001	0.0002	0.0001	0.0003
79	11	2608	KPARLVF	0.0003	0.0001	0.0002	0.0001	0.0003
79	11	1820	KPTLVGPTL	1.4150	0.0001	0.0002	0.0001	0.0003
79	11	1820	KPTLVGPTL	0.0021	0.0001	0.0001	0.0002	0.0002
93	13	1888	LPALSPGA	0.0001	0.0001	0.0001	0.0002	0.0002
93	13	1888	LPALSPGA	0.0053	0.0001	0.0036	0.0001	0.2100
86	12	1888	LPALSPGAL	0.0003	0.0002	0.0002	0.0002	0.0005
100	14	807	LPALSTGL	0.0020	0.0002	2.0000	0.0002	0.0005
86	12	887	LPALSTGLH	0.0350	0.0001	0.0001	0.0001	0.0002
86	12	887	LPALSTGLH	0.0011	0.0001	0.0001	0.0001	0.0002
86	12	2185	LPCEPEFV	0.0001	0.0002	0.0001	0.0001	0.0002
93	13	169	LPCEPEFV	0.0110	0.0360	0.0059	0.0150	0.0018
93	13	169	LPCEPEFV	0.1950	0.0790	0.0550	0.0013	0.0015
93	13	169	LPCEPEFV	0.0022	0.0009	0.0100	0.0140	0.0012
93	13	169	LPCEPEFV	0.0007	0.0001	0.0180	-0.0002	0.0020
93	13	37	LPCEPEFV	6.5000	0.0001	0.0009	0.0001	0.0025
93	13	37	LPCEPEFV	0.1900	0.0001	0.0002	0.0110	0.0003
93	13	1553	LPVCOOH	0.0005	0.0048	0.0002	0.0110	0.0003
86	12	1553	LPVCOOH	0.0001	0.0001	0.0002	-0.0002	0.0013
86	12	1553	LPVCOOH	0.0001	0.0001	0.0040	-0.0002	0.0013
86	12	1720	LPVCOOH	0.0011	0.0001	0.0002	0.0001	0.0003
100	14	1260	NPVAAITL	0.0011	0.0001	0.0002	0.0001	0.0002
100	14	1260	NPVAAITL	0.0001	0.0001	0.0001	0.0001	0.0001
86	12	1805	NPVAAITL	0.0003	0.0002	0.0001	0.0001	0.0002
86	12	1805	NPVAAITL	0.0002	0.0002	0.0001	0.0001	0.0002
79	11	1805	NPVAAITL	0.0001	0.0001	0.0001	0.0001	0.0001
79	11	1805	NPVAAITL	0.0001	0.0001	0.0001	0.0001	0.0001
79	11	2317	PPVHGPPL	0.0140	0.0001	0.0001	0.0001	-0.0002
79	11	2801	PPVHGPPL	0.0011	0.0001	0.0001	0.0002	0.0180
79	11	2801	PPVHGPPL	0.0002	0.0002	0.0002	0.0001	0.0002
79	11	2801	PPVHGPPL	0.0001	0.0002	0.0002	0.0001	0.0002
86	12	78	PPVHGPPL	0.0006	0.0002	0.0002	0.0001	0.0002

ICV B07 Super Motif (with Binding Information)

Conservancy	Freq.	Position	Sequence	B*0702	B*3501	B*5101	B*5301	B*5401
86	12	78	QPGVPMFLY	0.0001	0.0011	0.0002	0.0001	0.0002
93	13	57	QPGGRFOP	0.2300	0.0002	0.0001	0.0001	0.0002
79	11	2289	RFQVNPFL	0.0050				
93	13	1893	SPGALVGV	0.0001	0.0002	0.0002	0.1200	0.0002
79	11	1893	SPGALVGVW	0.0130	0.0001	0.0018	0.0001	0.0003
79	11	2931	SPGEINIV	0.0007				
79	11	2931	SPGEINIVA	0.0003	0.0001	0.0001	0.0002	0.0037
79	11	2649	SPQORVEF	0.0027				
79	11	2649	SPQORVEL	0.1200	0.0002	0.0002	0.0001	0.0002
79	11	89	SPRGSRPSW	0.3800	0.0002	0.0005	0.0001	0.0002
88	12	1935	SPTHVPESDA	0.0001				
86	12	1875	TPCSGSWL	0.0028				
79	11	1126	TPCTCGSSDL	0.0005				
79	11	1126	TPCTCGSSDLY	0.0001	0.0001	0.0002	0.0001	0.0003
86	12	223	TPGCVPV	0.0001				
93	13	1550	TPGLPVCOOHL	0.0001				
93	13	1627	TPLLYRLGA	0.0003				
93	13	1627	TPLLYRLGAV	0.0120	0.0001	0.0001	0.0002	0.2300
88	12	2656	TPVNSWLGNH	0.0001	0.0001	0.0008	0.0001	0.0110
86	12	2656	TPVNSWLGNH	0.0001	0.0001	0.0053	0.0006	0.0003
86	12	1940	VPESDAAA	0.0022				
88	12	1940	VPESDAARV	0.0001	0.0001	0.0010	0.0001	0.0003
86	12	799	WPLLLILL	0.0021				
100	14	616	YPYRLWHY	0.0001				

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HCV B27 Super Motif

Table XII

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy [%]
AKHMMWFI	1767	8	12	86
AKNEVFCV	2593	8	12	86
ARALAHGV	148	8	14	100
DRSELSPL	663	8	11	79
EKGGKPPA	2603	8	11	79
EKMALYDV	2624	8	12	86
FKOKALGL	1733	8	12	86
GHRMAWDM	315	8	13	93
GKSTKVPA	1240	8	12	86
GRKPARLU	2806	8	11	78
HRMAWDMM	316	8	13	93
KGGRRLI	1390	8	11	78
IRITGVRTI	1283	8	11	79
KKCDELA	1403	8	14	100
KKCDELA	1402	8	14	100
LHGPTPLL	1623	8	11	79
LHCNIVDV	697	8	12	86
LRLAVAV	889	8	11	79
NNVSPTHY	1832	8	12	88
PRGRRPRA	58	8	13	93
PRGSRPSW	100	8	11	79
PIRRISRL	112	8	12	86
RHADVIPV	1140	8	11	79
RHITPNSW	2854	8	12	86
RKLGWPLL	2943	8	12	86
RKPARLIV	2607	8	11	79
RICRASGV	2730	8	13	93
RPGFTLGV	38	8	12	86
RRFQDVKF	17	8	14	100
SKKCOEL	1401	8	12	86
SRNLGKVI	116	8	13	93
THIDAHFL	1571	8	12	86
TKLKLTPI	2885	8	12	86
TKVPAAYA	1243	8	14	100
TRCFDSTV	2674	8	11	79
TRGVAKAV	1181	8	14	100
VRNCEKMA	2620	8	14	100
VRMLEDGV	155	8	13	93
YRGLDVSV	1423	8	14	100
ARHTPNSW	2853	9	11	79
ARLNVFPL	2810	9	11	79
ARLVVLATA	1346	9	12	86
ARMLMTHF	2874	9	11	79
ARPDYNPPL	2298	9	11	79
DRSELSPL	663	9	11	79

HCV B27 Super Motif

Sequence	Position	Peptide No.	No. of Amino Acids	Sequence Frequency	Conservancy (%)
EKMALYDOW	2624		9	12	86
FKOKALGLL	1733		9	12	88
GHRMAWDMM	315		8	13	93
GKSTKVPAA	1240		8	12	86
GRKPARLV	2608		9	11	79
HRRMAWDMM	316		3	12	86
KGGRHLF	1390		8	11	79
KKKCOELAA	1402		8	14	100
LHGLSAPSL	2919		9	11	79
LHGPTPLLY	1623		9	11	79
LHSYSPGEI	2827		9	11	79
LKSSGGPL	1166		9	12	86
LKILGVPL	2942		9	12	86
NIWSPTHYV	1932		9	12	86
NRFPQWKF	16		9	11	79
PRGPRLG	38		9	13	93
PHTPVNSWL	2854		9	12	86
RVNGPGECA	1909		8	11	79
RKPARLVF	2607		9	11	79
RRCRASGVL	2730		9	12	86
RFSRLGKV	114		9	12	86
SKKKCOELA	1401		9	14	100
THYVPESDA	1937		9	12	88
TKVPAAYAA	1243		8	11	79
TRVADVIPV	1139		9	11	79
TRVESENKV	2251		9	12	86
VKFFGGGCI	22		9	13	93
VRVCEKML	2620		9	14	100
WRLAPITA	1028		9	11	79
WRQBWGGN	2242		9	12	86
YRGLDYSVI	1423		8	14	100
YRCRASGV	2728		9	13	93
ARALAHGVIV	148		10	14	100
ARAQAPPSPW	1600		10	11	79
ARRTPVNSWL	2853		10	11	79
ARMILMTHFF	2874		10	12	86
CSKQKODEL	1398		10	14	100
DRSELSPL	881		10	11	79
DRSELSPL	663		10	11	79
EXGGRRKPARL	2603		10	11	79
FRAAVCTRGV	1185		10	12	86
GHRMAWDMM	315		10	12	86
GKSTKVPAA	1240		10	12	86
GRKPARLV	2606		10	11	79
KHWNFRSGI	1768		10	13	93

HCY B27 Super Motif

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)
KKCDLAALK	1403	10	12	86
LHONNDVOY	697	10	11	79
LKSSGGRL	1168	10	12	86
OKALGLLTA	1735	10	12	86
RRNGPEGAV	1809	10	11	79
RRPRLGVRA	39	10	13	93
RRMGPEGA	1908	10	11	79
RRSRNLGKV	113	10	12	88
RRSRNLGKV	114	10	12	88
SKFGYGAQV	2552	10	12	86
SKKKCDLAA	1401	10	14	100
THYVPESDAA	1937	10	12	86
TRGVAKAVDF	1191	10	11	79
TRVESENKW	2251	10	12	86
VKPPGGQV	22	10	13	93
VTVCKKNALY	2620	10	14	100
VRLDGVNY	155	10	12	86
WRLAPITAY	1028	10	11	79
YKVLVLPVS	1254	10	14	100
YRRCRASGL	2729	10	12	86
YIGVRLDGV	152	11	13	93
YKHWNPISGI	1767	11	12	86
YRALAHGVRL	148	11	14	100
YRLVFPDLG	2810	11	11	79
CHSKKKCDLA	1399	11	14	100
DRDSELSRL	681	11	11	79
EXGGRKPARJ	2603	11	11	79
FRAAVCTRGVA	1185	11	11	79
GKSTKVPAAVA	1240	11	12	86
GKVIDLTGCF	120	11	12	86
HRMAWDMNNW	316	11	12	86
KKKCDLAALK	1402	11	12	86
KNTNRPPQGV	12	11	12	86
LHGPTLLYRL	1623	11	11	79
LHONNDVOYL	697	11	11	79
LKPTLHGFTPL	1619	11	11	79
LRRMGPEGA	1907	11	11	79
PRRGRPLGVRA	38	11	13	93
PRRSRLGKV	112	11	12	86
RRMGPEGAV	1908	11	11	79
RRSRNLGKV	113	11	12	86
SRGNNSPTHY	1929	11	12	86
SPRLGKVIDTL	116	11	12	86
THYVPESDAAA	1937	11	12	86
VRLDGVNYA	155	11	12	86

UCV B27 Super Motif

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)
YKVLVNPVA 136	1254	11	14	100

HCV B58 Super Motif Table XIII

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)
ANLRRHV	1804	8	13	93
ALAAYCL	1673	8	12	88
AAGCYKL	1250	8	11	79
ANLGFGA	1264	8	14	100
ANVCTRGV	1187	8	12	88
ASUMAFYA	1793	8	11	79
ASSASQL	2204	8	14	100
ATLFGAY	1285	8	14	100
CSFSRL	172	8	14	100
CSGGAYDI	1310	8	12	86
CSSNVSYA	2819	8	14	100
CTCGSSQL	1128	8	11	79
CTRGVAKA	1180	8	11	79
DTAACGDI	994	8	12	86
DTLCGFA	124	8	12	86
EALLENLY	750	8	11	79
EAMTRYSA	2794	8	14	100
ESDAARV	1942	8	12	86
ETAGARLV	1342	8	12	86
ETIMRSPV	1207	8	12	86
FADLMGYI	130	8	13	93
FASRGNTV	1927	8	14	100
FSIFLLAL	174	8	14	100
FSYDIRCF	2670	8	11	79
FTEAMTRY	2792	8	14	100
FTSPVW	512	8	13	93
GAGVAGAL	1861	8	12	86
GAHWGVLA	350	8	12	86
GALWGW	1895	8	11	79
GARLVLA	1345	8	12	86
GSGASTRV	1238	8	13	93
GSSDLTV	1131	8	12	86
GSSGFRLL	1188	8	12	86
GSSYGFQY	2641	8	11	79
GTFPINAY	2083	8	11	79
HSYSPGEI	2928	8	11	79
HTPNSWL	2855	8	12	86
ISGIQYLA	1774	8	14	100
ITSCSNV	2816	8	14	100
ITWGAOTA	989	8	12	86
KSTKVPAA	1241	8	12	86
LAGYGAGV	1857	8	11	79
LAHGVRL	151	8	14	100
LAVAVEPV	972	8	11	79
LSAPSLKA	2211	8	11	79

HCV B58 Super Motif

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)
LSPGALW	1892	8	13	93
LSTGLHL	690	8	12	86
LTCGFADL	126	8	12	86
LTHIDAF	1570	8	13	93
MSADLEV	1654	8	11	79
NSWLGNI	2869	8	14	100
NTCVTQTV	1460	8	12	86
NTGSGWH	416	8	13	93
PAILSPGA	1889	8	13	93
PALSTGLI	688	8	12	86
PTLWARIM	2870	8	11	79
PTLLYRL	1628	8	14	100
QATVCARA	1595	8	13	93
RAPRIMFM	3019	8	14	100
RSELPPL	564	8	11	79
RSPNLGV	115	8	12	86
SAFSUHSY	2923	8	11	70
SSASQLSA	2206	8	14	100
STKVPAAV	1242	8	12	86
STLPGNPA	1784	8	14	100
STLPQAVM	2633	8	12	86
STYQKFLA	1299	8	12	86
TACGDOI	995	8	12	86
TAGATILW	1343	8	12	86
TTMRSPVF	1208	8	12	86
TTCGNTL	2739	8	11	79
VAGALVAF	1884	8	12	86
VTRHADVI	1138	8	11	79
VTSTWVLV	1681	8	12	86
WAKHWNMF	1766	8	12	86
WAKVLIVM	368	8	14	100
WAOGYPM	78	8	12	86
YAOGYKY	1249	8	11	79
YSEPLDL	2805	8	11	78
YSTYKFL	1298	8	12	86
YTWDOOL	1106	8	11	79
AAKLODCTM	2758	9	16	114
AAOGYKLV	1250	9	11	78
AARALAHGV	147	9	11	79
AATLGFAY	1264	9	14	100
AAVCTRGA	1187	9	11	79
ASQLSAPSL	2208	9	13	83
ATLQFGAYM	1285	9	26	185
ATVCARAOA	1586	9	11	79
CAAILRRHV	1903	9	13	93

HCV NS5B Super Motif

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy [%]
CAWYELTPA	1530	9	11	78
CSFSLRA	172	9	14	100
CSGGAYDI	1310	9	12	86
CTCGSSDLY	1128	9	11	78
CTRGVAKAV	1190	9	11	78
CTWVNSTGF	555	2	11	79
DAGCAWYEL	1527	9	11	79
DTAACGDII	994	8	12	86
DTRCFDSTV	2873	8	13	93
ETAGARLW	1342	9	12	86
ETMTSPVVF	1207	9	12	86
FSRLALL	174	8	12	100
FSLDPTFI	1469	9	14	100
FTGLTHIDA	1567	9	13	93
GAGVAGALV	1861	9	12	88
GALVAFKIM	1866	9	12	86
GALVAFKVM	1866	9	14	100
GAVVWVWFL	1916	9	14	100
HSKKKDEL	1400	8	14	100
HTPCGVPCV	222	9	11	78
ITWGAOTAA	989	9	12	86
ITYSTYKGF	1296	9	12	88
KALGLLOTA	1736	9	12	86
KSTKVPAAV	1241	9	12	86
LAALAAAYCL	1672	9	12	86
LAQRFQKA	1729	9	12	86
LAGLAYYSM	356	9	14	100
LAGYGAGVA	1857	9	11	79
LSAFSLHSY	2922	9	11	79
LSTLPGNPA	1783	9	14	100
LTCGFADLM	126	9	24	171
LTDPSHITA	2180	9	14	100
LTFGRXNOV	1052	9	12	86
LTHIDAHFL	1570	8	13	93
LITSCGNTL	2738	9	11	79
MARNEVFCV	2592	9	12	86
MAVDMMMMW	318	8	12	86
NAVAYYRGL	1418	9	13	93
NSLRHNNM	2681	9	14	100
NSWLGNIM	2659	9	24	171
NINRRPOOV	14	9	12	86
PALSPGAL	1889	9	13	93
PSVAATLGF	1261	9	14	100
PTLHGPTFL	1621	9	11	79
PTLWARMIL	2870	9	11	79

HCV B58 Super Motif

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)
QAEIAGARL	1340	8	12	86
RAAVCTRGV	1186	8	12	86
RAIAHGVRV	148	8	14	100
RAQAPPSW	1601	9	11	79
RAYAMDREM	811	9	16	114
RSELKLL	664	2	11	79
RSNRLGKI	115	9	12	86
SSSASQLSA	2205	9	14	100
STKVPAAVA	1242	8	12	86
STLPGNPAL	1784	9	11	79
STWLVGGV	1663	9	12	88
TAGARLVVL	1343	9	12	86
TSCSSNSV	2817	9	14	100
TTIMAKNEV	2589	9	11	79
VAAITLGFCA	1263	9	14	100
VAGGHWOM	933	9	14	100
VAYOATVCA	1592	9	12	86
VAYVGLDV	1420	9	14	100
VSTLPQAVM	2832	9	12	88
VTOIVDFSL	1463	9	12	86
WAKHAWNFI	1768	9	12	88
YAAQGYRVL	1249	8	11	79
YAPTLWARIM	2868	9	14	100
YSPGENRV	2830	9	11	79
YSPQRMEF	2848	8	11	79
YSTYGRFLA	1298	8	12	86
YTNVDDLV	1106	9	11	79
YAOGYRVL	1250	10	11	79
YATLGFAYM	1264	10	28	186
ASLRVTEAM	2787	10	12	86
ASSASQLSA	2204	10	14	100
ATGMLPCSF	165	10	13	93
CSFSIFLAL	172	10	14	100
CTCGSSQLYL	1128	10	11	79
DARVCAQLWM	733	10	18	129
DSVIDCNTGV	1454	10	12	88
DTLTGFAOL	124	10	12	86
EANLLWROEM	2237	10	24	171
ETAGARLVVL	1342	10	12	86
FADLMGYFL	130	10	11	79
FTEANTRYSA	2792	10	14	100
GAARALAHQV	146	10	11	79
GADTAACGDI	992	10	12	86
QAGVAGALVA	1861	10	12	86
GALVVGVCVA	1895	10	11	79

HCY B58 Super Motif

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)
GARLVVLATA	1345	10	11	79
GAVQWNRRL	1816	10	14	100
QSGKSTKVP	1238	10	12	86
GTVLQDAETA	1335	10	14	100
HSKKQDELA	1400	10	14	100
IAFASRGNHV	1825	10	14	100
ISGIQYLAGL	1774	10	14	100
ITRVESENK	2250	10	12	86
ITSCSNVSV	2818	10	14	100
ITYSTYQKEL	1296	10	11	79
KSTKVPAAAYA	1241	10	12	86
LADGCSGGA	1305	10	11	79
LAEDFKOKAL	1729	10	12	86
LALPPRAYAM	806	10	12	86
LSPGALVGV	1892	10	13	93
LSRGRSPSW	88	10	11	79
LSRAPRPFM	3017	10	14	100
LSTLPGNPAI	1763	10	11	70
LTHPTIKYIM	1842	10	16	114
NTCVIQTVDF	1460	10	12	86
PAILSPGALV	1889	10	12	86
PALSTGLIHL	688	10	12	86
PARLVFPDL	2608	10	11	79
PSWDMWKKL	1807	10	11	79
PTGSGKSTK	1236	10	13	93
PTHVPESDA	1936	10	12	86
PTLHGPTPL	1621	10	11	79
PTLWARMILM	2870	10	22	157
PTLLYHLGA	1628	10	13	93
QAEAGARLV	1340	10	12	86
QAPFSSWDM	1603	10	24	171
QATVCARAQA	1595	10	11	79
RAAKLQDCTM	2757	10	16	114
RAAVCTRGVA	1188	10	11	79
RALAHGVRL	148	10	14	100
SASQLSPSL	2207	10	13	93
STKVPAAAYA	1242	10	11	79
STMVLGGVL	1663	10	12	86
TAGARLVVLA	1343	10	12	86
TARHTPNSW	2852	10	11	79
TSCSNNSVA	2817	10	14	100
TSMITOPSHI	2177	10	13	93
TSTMVLGGV	1682	10	12	86
TTIMAKNEVF	2589	10	11	79
TTLPALSTGL	685	10	11	79

IICV B58 Super Motif

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)
VAATLGFGAY	1263	10	14	100
VTPGERPSGM	1507	10	16	114
VTRHADVPV	1138	10	11	79
WAQGYWPL	76	10	12	86
WARMILATHF	2873	10	12	86
WAPDYNPL	2297	10	11	79
YAAGGYKLV	1249	10	11	79
YSPGENRVA	2930	10	11	79
YSPGQVEFL	2648	10	11	79
YARALHGRV	147	11	11	79
AASLRVFTAM	2788	11	12	86
AAVCTRGVAKA	1187	11	11	79
ASHLPYIEOGM	1717	11	14	100
ASOLSAPSLKA	2208	11	11	79
CARAGAPPSW	1599	11	11	79
CSFSIFLLAL	172	11	14	100
CTGSSDLYLV	1128	11	11	78
CTRGVAKAVDF	1190	11	11	79
DARVCACLWMM	733	11	16	114
DTLTGCFADLM	124	11	24	171
ETAGARLWLA	1342	11	12	86
FAOLMGYRLV	130	11	11	79
FSLSYSPGE	2925	11	11	79
FTGLTHDAHF	1567	11	13	93
FTLPALSTGL	884	11	11	79
GADTAACGDII	992	11	12	86
GAGVAGALVAF	1861	11	12	86
QALVVGVCAC	1895	11	11	78
GAVQWNRLLA	1818	11	14	100
GSGKSTRVPAA	1238	11	12	86
HSKKKCOELAA	1400	11	14	100
HSYSPGENRV	2928	11	11	79
HTPWNWLGW	2855	11	12	86
ITRVESENKV	2250	11	12	86
ITSCSNVSV	2816	11	14	100
ITYSTYGNFLA	1286	11	11	79
KSTRVPAAYAA	1241	11	11	79
LADGGGSGGAY	1305	11	11	79
LAGYGAGVAGA	1857	11	11	79
LSNSLRTHMM	2479	11	14	100
LSPGALVQGW	1892	11	11	78
LTCGFADLNGY	126	11	12	86
LTSMLTDFSHI	2178	11	13	93
NAVATYRGLDV	1418	11	13	93
NINFRPODNKE	14	11	11	79

HCV B58 Super Motif

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)
PAILSPGALVV	1889	11	12	86
PSVAATLGFGA	1261	11	14	100
PTDPRRSRNIL	109	11	12	86
PTHYVPESDAA	1936	11	12	86
PTLHGPTFLY	1621	11	11	79
PTPLLYRLGAV	1626	11	13	93
QAETAGARLVV	1340	11	12	86
QAPPSNDQMW	1603	11	11	79
QIVDFSLDPTF	1465	11	12	86
RSDPRGRORF	55	11	13	93
SADLEWTSTW	1655	11	11	79
SSASQLSAPSL	2206	11	13	93
SSDLYLVRHA	1132	11	12	86
STWVLGGVLA	1663	11	12	86
TARHTPVANSWL	2852	11	11	79
TSIGTRDKNQV	1050	11	12	86
TSTWVLGGVL	1662	11	12	86
TTLPALSTGL	685	11	11	79
VAATLGFQAYM	1283	11	26	106
VAGALVAFKVM	1864	11	14	100
VAVERVAFSDM	974	11	12	86
VAYQATVCARA	1592	11	11	79
VAYRGLDVSV	1420	11	14	100
VISTWVLGGV	1681	11	12	86
WADQGYWPLY	78	11	12	86
WARMILMTHFF	2873	11	12	86
YAAQGYKALVL	1249	11	11	79
YATQNI PQCSF	164	11	12	86
YTNDDQLVGW	1106	11	11	78

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HCY B62 Super Motif Table XIV

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)
AILSPGAL	1880	8	13	93
ALAHGVV	150	8	14	100
ALGLLOTA	1737	8	12	88
APTLWARM	2869	8	11	78
ADAPPPSW	1602	8	12	88
AGGKVLV	1251	8	11	79
AVAYRGL	1419	8	14	100
AVCTRGVA	1188	8	11	79
AVQWNRRL	1917	8	14	100
CLWMMILL	739	8	12	86
CASADLEV	1853	8	11	79
ODHLFW	1556	8	12	86
CVTOTVDF	1462	8	12	88
DILAGYGA	1855	8	12	86
DLOGSVRL	278	8	12	86
DLNGVPL	132	8	11	79
DLNLLPA	1883	8	11	78
DOAETAGA	1339	8	12	86
EIPFYGKA	1377	8	13	93
EOROKAL	1731	8	12	86
EWTSITWV	1659	8	12	88
FSGIOYL	1773	8	14	100
FPLGVV	2615	8	11	79
FGGQGV	24	8	14	100
FGVNLHA	1228	8	12	86
GIQYLAGL	1778	8	14	100
GLRDLAVA	988	8	11	79
QPTLGVRA	41	8	13	93
GONGGV	28	8	14	100
GVAGALVA	1863	8	12	86
GVAKAVDF	1193	8	11	78
GVLALAA	1670	8	12	86
GVRVCEGA	2518	8	14	100
GWCAAIL	1800	8	11	79
HVGFGEA	1910	8	11	79
HVSPTHW	1933	8	12	86
ILGGWAA	1816	8	12	86
ILGIGTVL	1331	8	12	86
ILSPGALV	1891	8	13	83
IMAXNEVF	2591	8	12	88
IPFYGKAI	1378	8	13	93
IPLVGARL	137	8	11	79
NOVOYLY	701	8	12	88
NPPDLGV	2613	8	11	79
NGGVILL	30	8	13	93

HCY B62 Super Motif

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)
KMALYDVV	2525	8	12	88
KPARLIVF	2608	8	12	88
KOKALGIL	1734	8	12	86
KVPAAYAA	1244	8	11	79
LIEANLLW	2235	8	12	86
LNTNGSW	414	9	11	79
LLALLSCL	178	8	12	86
LLAPITAY	1030	8	14	100
LLADARV	729	8	13	93
LLYRLGAV	1629	8	13	93
LMGYFLV	133	8	11	79
LPALSTGL	687	8	14	100
LPQSFSEI	169	8	13	93
LPRGPFRL	37	8	13	93
LPVDOOHL	1553	8	13	93
LPYEOGM	1720	8	12	86
LODCTMLV	2761	8	12	86
LVAYQATV	1691	8	12	86
LVQILAGY	1853	8	11	79
LVGGWLA	1687	8	12	86
LVNPSVA	1257	8	14	100
LVNLLPAI	1884	8	11	79
LVTRHADV	1137	8	12	86
LWGVNCA	1897	8	11	79
LWICESA	2773	8	11	79
MLMTTHFF	2878	8	12	86
MLTDPSHI	2178	8	14	100
NILGGWA	1815	8	12	86
NIVDVOTL	700	8	12	86
NLWFOEM	2239	8	12	86
NPSVAATL	1260	8	14	100
PLGGAARA	143	8	11	79
PLYRLGA	1628	8	13	93
PPSWDDMM	1605	8	12	86
PPSWDDMM	1608	8	11	79
PVHIGQPL	2318	8	11	79
QVGGVYL	29	8	13	93
QURIPQA	336	8	12	86
QPEYQLEL	2808	8	11	79
QKGYWFL	78	8	12	86
RLHLSAF	2918	8	12	86
RLVFPDL	2811	8	11	79
RLAPITA	1029	8	12	86
RLWLATA	1347	8	12	86
RMAYDMM	317	8	12	86

HCV B62 Super Motif

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)
RMILMTHF	2875	8	12	86
RPOVNPFL	2299	8	11	78
ROBMGGNI	2243	8	12	86
RVCCEJAL	2621	8	14	100
RVESEKIV	2252	8	12	86
RNGDRHYW	2100	9	11	79
SIFLLALL	175	8	14	100
SLDPTFTI	1470	8	14	100
SPGENRV	2931	8	11	79
SPGCRVEF	2849	8	11	78
SQLSNPSL	2209	8	13	93
SVAATLGF	1262	8	14	100
TIMAKNEV	2590	8	11	79
TLGFGAYM	1266	8	13	93
TLHPTPTL	1622	8	11	79
TLPGNPAL	1785	8	11	79
TLWARMIL	2871	8	11	79
TPCSGSWL	1876	8	12	86
TPGCVPCV	223	8	12	86
TOIVDFSL	1484	8	12	86
TVCARAGA	1597	8	11	79
VIDONTCV	1456	8	12	86
VLAAALAY	1871	8	12	86
VLDECYDA	1521	8	13	93
VLDQAEIA	1337	8	14	100
VLEGGNY	157	8	12	86
VLPFSVAA	1258	8	14	100
VLVGGLA	1686	8	12	86
VLVNFSV	1258	8	14	100
VMGSSYGF	2638	8	11	78
VPESDAAA	1940	8	12	86
VQMMNRLI	1918	8	14	100
VVATDALM	1439	8	11	79
WGWVCAA	1898	8	11	78
WVTSWVL	1860	8	12	86
WNNRLIAF	1920	6	14	100
WPLLIILL	798	8	12	86
WVLGGVL	1665	8	12	86
YLAGLSTL	1779	8	14	100
YPYRLWHY	616	8	14	100
YPESDAA	1938	8	12	86
ALSPGALV	1890	9	12	86
ALAHGVRL	150	9	14	100
ALSTGLHL	688	9	12	86
ALWGVVCA	1898	9	11	78

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)
APPSWDCM	1604	9	12	86
APTLWARI	2869	9	11	79
ADGYKLV	1251	9	11	79
AOPGYMFL	77	9	12	86
AVQWMNRL	1917	9	14	100
CMSADLEW	1653	9	11	79
DLOGSVRLV	279	9	11	79
DEWISTW	1657	9	12	86
DLNGYIPLV	132	9	11	79
DLVNLPLAI	1883	9	11	79
DLWICESA	2772	9	11	78
DLVLTIRHA	1134	9	12	86
DPQLSDGSW	2410	9	11	79
DPRESRL	111	9	12	86
EPFYGKAI	1377	9	13	93
EMGNITRV	2245	9	12	86
EWISTWNL	1659	9	12	86
FISGIQYLA	1773	9	14	100
FILLISCL	177	9	12	86
FILLADARV	728	9	13	93
FQYSPGQIV	2646	9	11	79
GIGTVLDOA	1333	9	14	100
GLPVODHL	1552	9	13	93
GLRDLAVAV	968	9	11	79
GLTHDAHF	1569	9	13	93
GFGEQAVQW	1912	9	12	86
GPTLLYRL	1625	9	14	100
GQVGGVNL	28	9	13	93
GVAGALVAF	1863	9	12	86
GVLAALAY	1670	9	12	86
GVNYATGNL	161	9	11	79
GVRVCEKMA	2619	9	14	100
GVRVLEDGV	154	9	13	93
HLHONMDV	696	9	12	86
HLPIECQGM	1718	9	11	79
HMWIFISGI	1769	9	13	93
HONNDVQY	598	9	11	79
HNGFEGAV	1910	9	11	79
ILAGYGAGV	1856	9	11	79
ILSPGALW	1891	9	13	93
KVLVNPVS	1255	9	14	100
LITSCSSNV	2815	9	14	100
LIVFPDLGV	2812	9	11	79
LIFLLADA	726	9	14	100
LIFNLGGW	1812	9	12	86

HCV B62 Super Motif (No binding data)

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)
UPRRGRL	36	9	13	83
LPAILSPGA	1888	9	13	93
LPALSTGL	887	9	12	86
LPCEPERDV	2165	9	12	86
LPGCCFSIF	169	9	13	93
LVGGVLAAL	1687	9	12	86
LVINPSVAA	1257	9	14	100
LVNLLPAL	1884	9	11	78
LVTRHADVI	1137	9	11	79
LWGVVCAA	1697	9	11	79
NILGGWAA	1815	9	12	86
NIRTGVRIT	1282	9	11	79
NVDVDTLY	700	9	12	86
NLGKVIDTL	118	9	12	86
NLPGCSFSI	168	9	13	93
NWDDLGVW	1108	9	11	79
PLGGAARAL	143	9	11	79
PLLYRLGAV	1628	9	13	93
PPPSWDDMMW	1605	9	11	79
PPWHGQRL	2317	9	11	79
POPEYDLEI	2807	9	11	78
PVDDHLEF	1554	9	12	86
PVNSHLGNI	2857	9	14	100
QWGGVYLL	29	9	13	93
QLSAPSLKA	2210	9	11	79
QPEYDLEI	2808	9	11	79
QPGYPMFLY	78	9	12	86
QPGPRQTI	57	9	13	93
RIILMITHFF	1028	9	12	86
RIILAPITAY	2875	9	12	86
RVCERMAALY	2621	9	14	100
RVESENKVV	2252	9	12	86
RVAEDGVNY	156	9	12	86
SMALTDPSSH	2178	9	14	100
SPGALVWGV	1893	9	13	93
SPGENRVA	2931	9	11	79
SPQORVERL	2649	9	11	79
SPGCRSPSW	89	9	11	79
SVDCNCTCV	1455	9	12	86
TMAKNEVF	2590	9	11	79
TLHGPTPLL	1622	9	11	79
TLPALSTGL	688	9	11	79
TLTCGFADL	125	9	12	86
TLWARMILM	2871	9	11	78
TLLYRLGA	1627	9	13	93

HCV B62 Super Motif

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)
TVLDOAETA	1336	9	14	100
VIDILTCGF	122	9	12	86
VLEDGWYA	157	9	12	86
VLVDILAGY	1852	9	11	78
VLVGGVLA	1666	9	12	86
VLVNPSPA	1256	9	14	100
VQWNNRLIA	1918	9	14	100
VGVVCAAI	1898	9	11	79
VVTSTWLV	1660	9	12	88
WNNRLIAFA	1920	9	14	100
WMLVGGVLA	1665	9	12	86
YIPLVGAPL	136	9	11	78
YLVAYCATV	1590	9	12	86
YLVTRHADV	1136	9	12	86
YQATVCARA	1594	9	13	93
YNGDQGSV	276	9	12	86
YNGVBFRL	637	9	13	93
YNPESDAAA	1939	9	12	86
YLSPGALVV	1890	10	12	86
ALVVGWCAA	1896	10	11	79
APPPSWDQW	1604	10	11	79
APTLLWARMIL	2869	10	11	79
AQPGYPWFLY	77	10	12	86
AVAYYRGLOV	1419	10	14	100
AVCTRGVAKA	1188	10	11	79
AVQWNNRLIA	1817	10	14	100
CLRLKGVPL	2941	10	12	86
CVTQTVDFSL	1462	10	12	86
DILAGYGAGV	1855	10	11	79
DLEVTSTW	1857	10	12	86
DLGVRCEQM	2617	10	13	93
QLSDGSWSTV	2412	10	11	79
DLVNLPLAIL	1883	10	11	79
DOAETAGARL	1339	10	12	86
DWFFGGGCI	21	10	12	86
ELITSCSSNV	2814	10	14	100
EDRKQKALGL	1731	10	12	86
EWSTWMLV	1659	10	12	86
GLSAFSLHSY	2921	10	11	78
GLSTLPGNPA	1782	10	14	100
GLTHDAHPL	1589	10	13	93
GFEGAGQWMA	1812	10	12	86
GQVGSYLL	28	10	13	93
GVCWTVYHGA	1091	10	11	79
GVRVCEKMAI	2619	10	14	100

HCY B62 Super Motif

Sequence	Position	Peptide No.	No. of Amino Acids	Sequence Frequency	Conservancy [%]
HQMDVOYL	698		10	11	79
ILAGYAGVA	1856		10	11	79
ILGGWAAQL	1816		10	12	86
IMAKNEVFCV	2591		10	11	79
IOYLAGLSTL	1777		10	14	100
NFRDLGVRV	2813		10	11	79
KPTLHGFTPL	1820		10	11	79
KVDTLTCGF	121		10	12	86
KVLVNPVA	1255		10	14	100
LLFNILGGW	1812		10	12	86
LLPAILSPGA	1887		10	13	93
LMGYIPLVGA	133		10	11	79
LPAILSPGAL	1888		10	13	93
LPGCSFSL	169		10	13	93
LPRGPRLV	37		10	13	93
LPVCDHLEF	1553		10	12	86
LVAYQATVCA	1591		10	12	86
LVOILAGYCA	1853		10	11	79
LVGGVLAALA	1867		10	12	86
LVGVVYCAAI	1897		10	11	79
MLIDPSHITA	2178		10	14	100
NIPGCSFSIF	168		10	13	93
NPSVAATLGF	1260		10	14	100
PITYSTYGNF	1285		10	11	79
PLGGAARALA	143		10	11	79
POPEYDLBJ	2807		10	11	78
PVCDHLEPV	1554		10	12	86
PVNSWLGMI	2857		10	14	100
PVYCFTPSPV	508		10	13	93
QIPCEPEFQV	2164		10	12	88
QPEKGGKQPA	2801		10	11	79
RHGLSAFSL	2818		10	11	78
RLVFPOLGV	2811		10	11	79
RLMAWDMMNNW	317		10	12	86
RLVEDGWNYA	158		10	12	86
SLHSYSPGEI	2826		10	11	79
SLTRDKNOV	1051		10	12	86
SPGALVVGW	1893		10	11	78
SOLSAPSLKA	2209		10	11	79
SOPTGRROFI	56		10	13	93
SVAAATLGGA	1262		10	14	100
TLHGFTPLLY	1822		10	11	79
TLFNILGGW	1811		10	12	86
TLPALSTGLI	686		10	11	79
TLTCGFADLM	125		10	12	86

HCY B62 Super Motif

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)
TPGTGSSDL	1126	10	11	79
TPLLYRLGAV	1627	10	13	93
TPVNSMLGNI	2856	10	12	88
TVDFSLDTIF	1486	10	12	86
VIOTLTGFA	122	10	12	86
VLAALAYCL	1671	10	12	86
VLDQAEATAGA	1337	10	12	88
VLNPSVAATL	1258	10	14	100
VLTTSCGNTL	2737	10	11	79
VLVGGVLAAL	1666	10	12	86
VLVLNPSVAA	1256	10	14	100
VMSSSYGFOY	2639	10	11	78
VPESDAARV	1940	10	12	86
VQWMNIIJAF	1818	10	14	100
VGVVVCAIL	1898	10	11	79
WLVGGVLAAL	1665	10	12	86
YLVGSSGGFL	1165	10	12	86
YLLPRRGPRL	36	10	13	92
YLVRHADVI	1136	10	11	79
YNGLDGSVF	278	10	12	86
ALVVGWVCAAI	1898	11	11	78
APTSGSKTKV	1235	11	13	93
APTLWARMILM	2869	11	11	79
AOAPPSWDOAM	1602	11	12	86
AVCTRGVAKAV	1186	11	11	79
AVQWMNIIJAF	1917	11	14	100
DILAGYGAGVA	1855	11	11	79
DLWTSTWNL	1657	11	12	86
DLGWRVCEKAA	2617	11	13	83
DLVGYRLVGA	132	11	11	78
DLVLRHADV	1134	11	12	86
DOAETAGARLV	1539	11	12	86
DMKFRGGGV	21	11	12	86
EGRKQKALGLL	1731	11	12	86
FSGDIYLAEL	1773	11	14	100
FLAGGCSGGA	1304	11	11	79
FRGGGNGGV	24	11	14	100
FOYSPGQRIEF	2646	11	11	79
GYTLAGLSTL	1778	11	14	100
GLPVQDHLLEF	1552	11	12	86
GLSTLPGNPAI	1782	11	11	78
GPITPLLYRLGA	1625	11	13	93
GPVVCFTPSFV	507	11	13	93
GVLAALAYCL	1670	11	12	86
GVRCERKUALY	2618	11	14	100

HCV H62 Super Motif

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)
GVRLEDGVNY	154	11	12	86
HLHNVDOY	696	11	11	79
HMNIFSGDY	1769	11	13	83
HONNDVOTLY	898	11	11	79
HVGPGEAVQW	1910	11	11	79
ILGGWAAQLA	1510	11	12	86
ILGIGTVLDOA	1331	11	12	86
ILSPGALWGV	1891	11	13	93
KPARLVFPDL	2608	11	11	79
KPTLHGPTLL	1620	11	11	79
KOKALGLOTA	1734	11	12	86
KVIDLTGFA	121	11	12	86
KVLVLPVAA	1255	11	14	100
LAFASRGNN	1924	11	14	100
LITSCSNWV	2815	11	14	100
LVPFOLGVR	2612	11	11	79
LLFLLADARV	726	11	13	93
LLFNILGWVA	1812	11	12	86
LLPALSPGAL	1887	11	13	83
LLPRRGPFLLV	36	11	13	79
LLSPRGSPSW	87	11	11	86
LLWRDEAGGV	2240	11	12	86
LPAILSPGALV	1888	11	12	86
LPALSTGLHL	687	11	12	86
LPGCSFSILL	169	11	13	93
LPQCHLEPW	1553	11	12	86
LVGGVLAALAA	1667	11	12	86
LVNPSVAATL	1257	11	14	100
LVTRHADVPV	1137	11	11	79
LVGVVVCNAIL	1887	11	11	78
NILGGWAAQL	1815	11	12	88
NITRVESNKV	2249	11	12	86
NLLPALSPGA	1688	11	13	93
NLPGCSFSIL	168	11	13	93
PITYSTYQKFL	1285	11	11	79
PLEGFGPDL	2403	11	11	93
PMGFSYDITCF	2687..	11	11	79
PPSWDQWKKL	1606	11	11	79
PVNSWLNHIM	2857	11	12	86
PVYCFIPSPW	508	11	13	83
RAWVGVEFL	635	11	13	93
RCMGGMITRY	2243	11	12	88
RVCENALYDV	2821	11	12	86
SIFLLALLSCL	175	11	12	86
SMLTDPSHITA	2178	11	14	100

HCY B62 Super Motif

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)
SPTHYPESDA	1835	11	12	86
SQPCPEPQV	2163	11	12	86
SVATLGFQAY	1262	11	14	100
TLGFGATMSKA	1266	11	12	86
TLLENLGGWV	1811	11	12	86
TPCTGSSDLY	1128	11	11	79
TPGLPVCOOIL	1550	11	13	83
TPVNSWLGNI	2856	11	12	86
TVLDQAEATAGA	1336	11	12	86
VLOECYDAGCA	1521	11	11	79
VLDVILAGYGA	1852	11	11	79
VLYGGVLAALA	1866	11	12	86
VQPEXGGRKPA	2600	11	11	79
VQWNNRLIAPA	1918	11	14	100
WCAAILRIRHV	1901	11	11	79
WVLVGGVLAAL	1665	11	12	86
YKGSSEGPLL	1165	11	12	86
YLVAQATVCA	1590	11	12	86
YQATVCARAQA	1594	11	11	79
YNGLOGSVRL	278	11	12	86
YWPESDAARV	1939	11	12	86

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Table XV HCY A01 Motif with Binding Information

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)	A*0101
ASFGSPY	166	8	20	100	
DNWLSRKY	737	10	18	90	0.0001
FAAFTOCGY	631	10	19	95	0.0880
GFAAFTOCGY	630	11	19	95	
GRETVEY	140	8	15	75	
GYSNFMGY	579	9	17	85	
HTLWKAGILY	149	10	20	100	
KOAFIFSPTY	653	10	19	85	0.1100
LDDTASALY	30	9	17	85	0.0001
LSLOVSAIFY	415	10	18	95	12.0000
LTFGRETVLEY	137	11	15	75	0.0150
MMWYWGPSLY	360	10	17	85	
MSTTLEAY	103	9	15	75	0.0810
NSWLSRKY	738	9	18	90	0.8500
PLDKGKPY	124	9	20	100	0.0005
PLDKGKPY	124	10	20	100	
PTTGRTSLY	797	9	17	85	0.1700
SASFGSPY	165	9	20	100	0.2100
SLOVSAIFY	416	9	19	95	
SITDLEAY	104	8	15	75	5.2000
TTGRTSLY	798	8	17	85	
WLSLOVSAIFY	414	11	19	95	
WMMWYWGPS	359	11	17	85	0.3200
YPALMPLY	640	0	19	95	
YSLNFMGY	580	0	17	85	

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Table XVI
UCV A03 Motif with Binding Information

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)	A*0301
AACNMTGER	647	10	12	88	0.0003
AARALAHGVR	147	10	11	79	
AATLGFGA	1264	8	14	100	
AATLGFGAY	1264	9	14	100	
AAVCTRGVA	1187	9	11	79	0.0003
AAVCTRGVAK	1187	10	11	79	
AAVCTRGVAKA	1107	11	11	79	
ACNMTGER	648	9	12	86	
ADGSCSGA	1308	9	11	79	0.0003
ADGGCSGGAY	1308	10	11	79	
ADVIPVRR	1142	8	12	86	
ADVIPVRRR	1142	9	11	79	
AFASRGNH	1926	8	14	100	0.0003
AGALVAFK	1865	8	12	86	
AGARLVVLA	1344	9	12	86	
AGARLVLATA	1344	11	11	79	
AGLSTLPNPA	1781	11	14	100	0.0003
AGVAGALVA	1862	9	12	86	
AGVAGALVAF	1862	10	12	86	
AGVAGALVAFK	1862	11	12	86	
AGWLLSPR	94	8	12	86	0.0003
AGWLLSPRGR	94	11	12	86	
AGYGAGVA	1058	8	12	86	
AGYGAGVAGA	1058	10	12	86	
ALGLLQTA	1737	8	12	86	0.0003
ALSTGLIH	609	8	12	86	
ALSTGLIHLH	609	10	12	86	
ALVGVWCA	1096	9	11	79	
ALVGVWCAA	1896	10	11	79	0.0003
ASLMAFTA	1793	8	11	79	
ASLSAPSLK	2208	10	11	79	
ASLSAPSLKA	2208	11	11	79	
ASRGNHVSPTH	1928	11	12	86	0.0003
ASSASOLSA	2204	10	14	100	
ATGNLPGCSF	165	10	13	93	
ATLGFGAY	1265	8	14	100	
ATLGFGAYMSK	1265	11	12	86	0.0003
ATRKTSER	48	8	11	79	
ATVCARAOA	1596	9	11	79	
AVCTRGVA	1188	8	11	79	
AVCTRGVAK	1188	9	11	79	0.0003
AVCTRGVAKA	1188	10	11	79	
AVQWMNRLLA	1917	10	14	100	
AVQWMNRLLAF	1917	11	14	100	
CAALIRRH	1903	8	13	93	

HCY A03 Motif with Binding Information

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)	A*0301
CAWYELTPA	1530	9	11	79	
CGFADLMGY	120	9	13	93	
CGNTLTGY	2742	8	11	79	
CGSSDLYLVR	1130	11	11	79	
CGYRRCRA	2727	8	14	100	
CLRKLGVFFLR	2941	11	12	86	
CSFSIFLLA	172	9	14	100	
CSSNVSA	2819	8	14	100	
CSSNVSAH	2819	9	12	86	
CTCGSSLY	1128	9	11	79	0.0001
CTRGVAKA	1190	8	11	79	
CTRGVAKAVDF	1190	11	11	79	
CTWVNSTGF	555	9	11	79	
CTWVNSTGFTK	555	11	11	79	
CVQPEKGGR	2599	9	11	79	0.7600
CVQPEKGGRK	2589	10	11	79	0.0000
CYTQTVDF	1462	8	12	86	0.0011
DAHFLSOTK	1574	9	14	100	
DOLWICESA	2771	10	11	79	0.0003
DFSLOTF	1460	8	14	100	
DGGSOSGA	1307	8	11	79	
DGGSOSGAY	1307	9	11	79	
DIIQDECH	1310	9	12	86	
DILAGYGA	1855	8	12	86	
DILAGYGAGVA	1055	11	11	78	
DLGVRVCEK	2617	8	13	93	0.0003
DLGVRVCEKMA	2617	11	13	93	
DLMGVPLVGA	132	11	11	79	
DLVNLPA	1883	8	11	79	
DLVVICESA	2772	9	11	79	
DLVLTTH	1134	8	12	86	
QLVLTTHA	1134	9	12	86	0.0003
DLTLCGFA	124	8	12	86	
DVPIVRRH	1143	8	11	79	
EAMTRYSA	2784	8	14	100	
ECYDAGCA	1524	8	11	79	
ECYDAGCAWY	1524	10	11	79	
EOLVNLPA	1882	9	11	79	
EGAVNNNR	1815	9	14	100	0.0004
EIPFYGA	1377	8	12	86	
EMGNTR	2245	8	12	86	
ETAGARLVLA	1342	11	12	86	
ETMRSPVF	1207	9	12	86	
EVQVQPEK	2586	9	12	86	0.0008
FOVQPEKGGR	2588	10	11	79	

HCV Δ03 Motif with Binding Information

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)	A*0301
FOVPEKGGPK	2590	11	11	79	
FGAYMSKA	1269	8	12	86	
FGAYMSKAH	1269	9	12	86	
FGCTWVNSTGF	553	11	11	78	
FGYCAKQVR	2554	9	12	86	0.0008
FISGIOYLA	1773	9	14	100	
PLADGGCSGQA	1304	11	11	79	
FLLADAR	728	8	14	100	
FSYDTRCF	2670	8	11	79	
FTEAMTRY	2792	8	14	100	
FTEAMTRYSA	2792	10	14	100	
FTGLTHIDA	1567	9	13	93	
FTGLTHIDAH	1567	10	13	93	
FTGLTHIDAHF	1567	11	13	93	
GAARALAI	146	0	11	79	
GAARALAHGVR	146	11	11	79	
GAGVAGALVA	1861	10	12	86	
GAGVAGALVAF	1861	11	12	86	
GAHWGLA	350	8	12	86	
GALVVGWCA	1895	10	11	79	
GALVVGWVCAA	1895	11	11	79	
GARLVLA	1345	8	12	86	
GARLVVLATA	1345	10	11	79	
GAVQWNR	1916	0	14	100	
GAVQWNRILJA	1918	11	14	100	
GAYMSKAH	1270	0	12	86	
GCAWYELTPA	1529	10	11	79	
GCSFSILLA	171	10	14	100	
GCTWVNSTGF	554	10	11	79	
GDDLWICESA	2770	11	11	79	
GLOGSVF	278	8	12	86	
GFADLMGY	129	8	13	93	
GFGAYMSK	1268	8	12	86	
GFGAYMSKA	1268	9	12	86	
GFGAYMSKAH	1268	10	12	86	
GFOYSPQQR	2645	9	11	79	
GFSYDTRCF	2689	9	11	79	
GGATIALA	145	8	11	79	
GGAAHALAH	145	9	11	79	
GGCSGGAY	1300	8	11	79	
GGGVGGWY	26	10	14	100	
GGHYQMA	935	8	11	79	
GGQVGGWY	27	9	14	100	
GGRIJFQI	1382	9	14	100	
GGRIJFQISK	1382	11	14	100	0.0003

HCV A03 Motif with Binding Information

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)	A*0301
GGKPARLIVF	2005	11	11	79	
GGVLAALA	1869	8	12	86	
GGVLAALAA	1669	9	12	86	
GGVLAALAA	1668	10	12	86	
GGVLLPR	32	8	13	93	
GGVLLPR	32	9	13	93	0.0003
GGWAAQLA	1818	9	12	86	
GIGVLDQA	1333	9	14	100	
GIYLPNR	3037	8	11	79	
GLPVCDH	1552	8	13	93	
GLPVCDHLEF	1552	11	12	86	
GLPVSARR	1004	0	11	79	
GLROLAVA	988	8	11	79	
GLSAFSLH	2921	0	11	79	
GLSAFSLISY	2921	10	11	79	0.0100
GLSTLPGNPA	1782	10	14	100	
GLTHIDAH	1569	8	13	93	
GLTHIDAHF	1569	9	13	93	
GSGKSTKVPAA	1238	10	12	86	
GSGKSTKVPAA	1238	11	12	86	
GSSDLVLTIR	1131	10	12	86	
GSSDLVLTIRH	1131	11	12	86	
GSSYGFY	2641	8	11	79	
GTFPINAY	2063	8	11	79	
GVLDONETA	1335	10	14	100	
GVAGALVA	1863	0	12	86	
GVAGALVAF	1863	9	12	86	
GVAGALVAFK	1863	10	12	86	0.3800
GVAKAVDF	1193	0	11	79	
GVQWTVYH	1081	8	11	79	
GVQWTVYHGA	1081	10	11	79	
GVGYLPNR	3035	10	11	79	0.0014
GVLAALAA	1670	8	12	86	
GVLAALAA	1670	9	12	86	0.0046
GVRATRTSER	45	11	14	100	
GVRVCEKMA	2619	9	14	100	
GVRVCEKMA	2619	11	14	100	
GVVLEDGWY	154	11	12	86	
GVVCAAILR	1900	9	11	79	
GVVCAAILRR	1900	10	11	79	
GVVCAAILRRH	1900	11	11	79	
GVYLLPRR	33	8	13	93	
GVYLLPRRPPR	33	11	13	93	
HADVIPVR	1141	8	11	79	
HADVIPVR	1141	9	11	79	

HCV A03 Motif with Binding Information

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)	A*0301
HADVIVRRR	1141	10	11	79	
HAPTGSQK	1234	8	14	100	
HAPTGSQSTK	1234	11	13	93	
HGLSAFSUH	2920	9	11	79	
HGLSAFSUHSY	2920	11	11	79	
HGPTLLY	1624	8	11	79	
HGPTLLYR	1624	9	11	79	
HIDAHLSQTK	1572	11	14	100	
HUHAPTGSQK	1232	10	12	86	0.5900
HJHQNVOVOY	696	11	11	79	
HLFCHSK	1395	0	14	100	
HLFCHSKK	1395	0	14	100	0.0260
HLFCHSKKK	1395	10	14	100	0.0260
HMMNFSGIQY	1769	11	13	93	
HSKKKCELA	1400	10	14	100	
HSKKKCELA	1400	11	14	100	
HSYSGEINR	2928	10	11	79	
HTPGVPCVR	222	10	11	79	0.0004
HMGPEGA	1810	0	11	79	
IAFASRGNH	1925	9	14	100	0.0003
IDAHLSTQK	1573	10	14	100	
IDTLTCGF	123	8	12	86	
IDTLTCGFA	123	9	12	86	
IFCHSKK	1397	8	14	100	
IGTVLOOA	1334	0	14	100	
IGTMLDOAETA	1334	11	14	100	
IICDECH	1317	8	12	86	
ILAGYGAGVA	1056	10	11	79	
ILGGWAA	1816	8	12	86	
ILGGWAAQLA	1816	11	12	86	
ILGIGTVLOOA	1331	11	12	86	
IMAKNEVF	2591	8	12	86	
ISGIOTLA	1774	8	14	100	
ITRVESENK	2250	9	12	86	0.0150
ITSCSNVSV	2816	11	14	100	
ITWGADTA	989	8	12	86	
ITWGADTAA	989	9	12	86	
ITYSTYCK	1296	8	12	86	
ITYSTYCKF	1296	9	12	86	
ITYSTYCKFLA	1296	11	11	79	
NDVOYLY	701	8	12	86	
NFPLGV	2613	9	11	79	0.0036
NGGMYLPR	30	10	13	83	0.0008
NGGMYLPRR	30	11	13	93	
KALGLLOTA	1736	9	12	86	

HCV A93 Motif with Binding Information

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)	A*0301
KDELAAK	1404	8	12	86	
KFGYGADNR	2553	10	12	86	
KGGRILIF	1391	8	11	79	
KGGRILFCH	1391	10	11	79	
KGGRKPAR	2604	8	11	79	
KLGVPLR	2944	8	12	86	
KSTKVPAA	1241	9	12	86	0.0008
KSTKVPAAAY	1241	10	12	86	
KSTKVPAAAYA	1241	11	11	79	
KSTKVPAAAYAA	1241	11	12	86	
KTKRNTNR	10	8	12	86	
KTKRNTNRH	10	9	12	86	0.0110
KTSESRPR	51	9	13	93	0.1600
KTSESRPRGR	51	11	12	86	
KVIDLTGCF	121	10	12	86	
KVIDLTGFA	121	11	12	86	
KVLVLPNSVA	1255	10	14	100	
KVLVLPNSVAA	1255	11	14	100	
KVPAAYAA	1244	8	11	79	
LADGCGSGGA	1305	10	11	79	
LADGCGSGGAY	1305	11	11	79	
LAOFKOK	1729	8	12	86	
LAOFKOKA	1729	9	12	86	
LAGYGAGVA	1857	9	11	79	
LAGYGAGVAGA	1857	11	11	79	
LCEGYDAGCA	1522	10	12	86	
LDQMETAGA	1338	9	12	86	
LDQMETAGAI	1338	10	12	86	
LFLLADA	727	8	14	100	
LFLLADAR	727	9	14	100	
LFNLLGWVA	1813	10	12	86	
LFNLLGGWVAA	1813	11	12	86	
LFESPRR	290	8	11	79	0.0810
LFGAYMSK	1267	9	12	86	
LFGAYMSKA	1267	10	12	86	
LFGAYMSKAH	1267	11	12	86	
LGAARALA	144	9	11	79	
LGAARALAH	144	10	11	79	
LGGWAAOLA	1817	10	12	86	
LIGTVLDOA	1332	10	13	93	
LGVRAIRK	44	8	12	86	
LGVRCCK	2618	8	14	100	
LGVRCCKMA	2618	10	14	100	
LIAFASRGNH	1924	10	14	100	
LIEANLLWR	2235	9	12	86	0.0008

HCV A03 Motif with Binding Information

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)	A*0301
LFOHSKK	1390	8	14	100	
LIFCHSKKK	1396	9	14	100	0.5400
LINTNGSWH	414	9	11	79	
LVPFDLGVK	2612	10	11	79	0.0003
LLAPITAY	1030	8	14	100	
LLFLLADA	726	9	14	100	0.0016
LLFLLADAR	726	10	14	100	
LLFNILGGWA	1812	11	12	88	
LLPAILSPGA	1887	10	13	93	0.0003
LLPRRGPR	36	8	13	93	
LLSPRGSR	97	8	12	86	
LMGYPLVGA	133	10	11	79	
LSAFSLHSY	2922	9	11	79	0.0002
LSAPSLKA	2211	8	11	79	
LSNSLRH	2479	9	12	86	
LSNSLRHH	2479	9	12	86	0.0003
LSTGLHLH	690	9	12	86	
LSTLPGNPA	1793	9	14	100	
LTCGFADLMGY	126	11	12	86	
LYDPSSHITA	2180	9	14	100	
LTHDAHF	1570	8	13	93	
LTSMLTOPSH	2176	10	13	93	
LVAYQATVCA	1591	10	12	86	
LVAYQATVCAR	1591	11	11	79	
LVDLIAGY	1053	0	11	79	
LVDLIAGYGA	1053	10	11	79	
LVGGVLA	1667	0	12	86	
LVGGVLAALA	1667	10	12	86	
LVGGVLAALAA	1667	11	12	86	
LVLPNSVA	1257	9	14	100	
LVLPNSVAA	1257	8	11	79	
LVVGWCA	1897	9	11	79	
LVVGWCAA	1897	8	11	79	
LVVCESA	2773	8	11	79	
MGFSYDTR	2668	8	11	79	
MGFSYDTRCF	2668	10	11	78	
MGSSYGFOY	2640	9	11	79	
MGYPVLGA	134	9	11	79	
MILMTHFF	2876	8	12	86	
MLTDPSSHITA	2179	10	14	100	
MSTNPKPOR	1	9	11	79	
MSTNPKPOR	1	10	11	79	
NOGYRRCR	2726	8	11	79	
NOGYRRCRA	2726	8	11	79	
NCSYRCH	305	8	11	79	

HCV A93 Motif with Binding Information

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)	A*0301
NFISQIY	1772	8	14	100	
NFISGIQYLA	1772	10	14	100	
NGVQWIVY	1080	8	11	79	
NGVQWTVYH	1080	9	11	79	
NGVQWTVYHGA	1080	11	11	79	
NILGGWA	1815	8	12	86	
NILGGWAA	1815	9	12	86	
NITRVESEK	2248	10	12	86	0.0010
NIVDVQYLY	700	9	12	86	0.0005
NILLPAILSPGA	1806	11	13	93	
NILPGCSFSIF	168	10	13	93	
NTCVTQTVDY	1460	10	12	86	
NINRPQDVK	14	10	11	79	
NINRPQDVKF	14	11	11	79	
NINRPQDVQDH	1549	11	13	93	
PAILSPGA	1089	8	13	83	
PALSTGLIH	688	9	12	86	
PALSTGLIHLH	688	11	12	86	
PCSGSWLR	1876	8	11	79	
PCTCGSSDLY	1127	10	11	79	
PDLGRVCEK	2616	10	13	93	
PGALVVGWCA	1894	11	11	79	
PGCSFSIF	170	8	14	100	
PGCSFSIFLLA	170	11	14	100	
PGGVPCVR	224	0	12	86	
PGGVQWMMNR	1913	11	13	93	
PGEINRVA	2932	0	11	79	
PGERPSGWF	1509	8	12	86	
PGGGGNGGVY	25	11	14	100	
PGLPVQDCH	1551	9	13	93	
PGYPWFLY	78	8	14	100	
PITYSTYK	1285	9	11	79	
PITYSTYKGF	1285	10	11	79	
PLGGAARA	143	8	11	79	
PLGGAARALA	143	10	11	79	
PLGGAARALAH	143	11	11	79	
PLYRLGA	1628	8	13	93	
PMGFSYDTR	2667	9	11	79	
PMGFSYDTRCF	2667	11	11	79	
PSPWVGTTDR	514	11	13	93	
PSVAATLGF	1261	9	14	100	
PSVAATLGFGA	1261	11	14	100	
PSWQMMK	1607	8	11	79	
PTDCFRKH	507	8	13	93	
PTDPRRPRSR	109	9	12	86	0.0008

ICY A03 Motif with Binding Information

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)	A*0301
PTGSGKSTK	1236	9	13	93	0.0002
PTHVPESDA	1936	10	12	86	
PTHVPESDAA	1936	11	12	86	
PTLHGPTFLY	1621	11	11	79	
PTPLLYRLGA	1626	10	13	93	
PVODDHEF	1554	9	12	86	
PWVGTTDR	516	9	13	93	0.0008
QAEAGAR	1340	8	12	86	
QATVCARA	1595	8	13	93	
QATVCARAQA	1595	10	13	93	
QNGGVLLPR	29	11	13	93	
QLFTSPR	209	0	12	86	
QLFTSPRR	209	9	11	79	0.7500
QLLNPOA	236	0	12	86	
QLSAPSLK	2210	8	11	79	
QLSAPSLKA	2210	9	11	79	
QTVDFSLDPTF	1465	11	12	86	
RAAVCTRGVA	1186	10	12	86	
RAAVCTRGVAK	1186	11	11	79	
RALAHGVR	149	8	14	100	
RATKTSER	47	9	11	79	
RGNVSPTH	1930	9	12	86	0.0003
RGNVSPTHY	1930	10	12	86	0.0003
RGPTLQVR	40	0	13	93	
RGPRLGVRRA	40	9	13	93	
RGPRLGVRATR	40	11	11	79	0.0120
RGPRROPK	59	9	13	93	
RGSLSPR	1154	8	12	86	
RGVAKAVDF	1192	9	11	79	
RLGVRATR	43	8	11	79	
RLGVRATR	43	9	11	79	0.9400
RLHGLSAF	2918	8	12	86	
RLHGLSAFSLH	2918	11	11	79	
RLIAFASR	1923	8	14	100	
RLIAFASRGNH	1923	11	14	100	
RLVFPQLGVR	2611	11	11	79	
RLAPITA	1029	0	12	86	
RLAPITAY	1029	9	12	86	2.7000
RLVVLATA	1347	8	12	86	
RLMLMTHF	2075	8	12	86	
RLMLMTHFF	2875	9	12	86	
RLVVGVEH	635	9	14	100	
RLVVGVEHR	635	10	14	100	0.7200
RSOPRGR	55	8	13	93	
RVCEKMALY	2621	9	14	100	0.1800

HCY A03 Motif with Binding Information

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)	A*0301
RLEDGVNY	156	8	12	86	0.0120
RLEDGVNYA	156	10	12	86	
SAFSLHSY	2023	8	11	79	
SASQLSAPSLK	2207	11	11	78	
SCSSNVSA	2016	9	14	100	
SCSSNVSAH	2818	10	12	86	
SDLYLVTR	1133	8	12	86	
SDLYLVTRH	1133	9	12	86	
SOLYLVTRHA	1133	10	12	86	
SFSIFLLA	173	8	14	100	
SGKSTKVP	1239	9	12	86	
SGKSTKVPAA	1239	10	12	86	
SGKSTKVPAAAY	1238	11	12	86	
SMLTDPHSI	2178	8	14	100	
SMLTDPSHITA	2178	11	14	100	
SSASQLSA	2206	8	14	100	
SSDLVTVTR	1132	9	12	86	0.0003
SSDLVTVTRH	1132	10	12	86	0.0003
SSDLVTVTRHA	1132	11	12	86	
SSNVSAH	2820	8	14	100	
SSASQLSA	2205	9	14	100	
STGLHHL	691	8	12	86	
STKVPAAAY	1242	9	12	86	
STKVPAAAYA	1242	9	12	86	
STKVPAAAYAA	1242	10	11	79	
STLPGNPA	1704	8	14	100	
STNPKPOR	2	8	11	79	
STNPKPORIK	2	9	11	79	
STNPKPORIKTK	2	11	11	79	
STWMLVGGVLA	1653	11	12	86	
STYGKFLA	1299	8	12	86	
SVAATLGF	1262	8	14	100	
SVAATLGFGA	1262	10	14	100	
SVAATLGFAGAY	1262	11	14	100	
TAGARLWLA	1343	10	12	86	
TCGFADLMGY	127	10	13	93	
TCGSSQLY	1128	8	11	79	
TCVTQTVDIF	1461	9	12	86	
TDPRRSR	110	8	12	86	
TOPSHITA	2101	8	14	100	
TGEIPFYGK	1375	9	11	79	
TGEIPFYGKA	1375	10	11	79	
TGLTHIDA	1568	8	13	93	
TGLTHIDAH	1568	9	13	93	0.0003
TGLTHIDAHF	1568	10	13	93	

ICV A03 Motif with Binding Information

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)	A*0301
TGMPOCSF	166	9	13	93	
TGSKSTK	1237	8	13	93	
TGSKSTKVP	1237	11	12	86	
TIMAKNEVF	2590	9	11	79	
TLGFAYMSK	1268	10	12	86	0.0810
TLGFAYMSKA	1268	11	12	86	
TLHGPTLLY	1622	10	11	79	0.0890
TLHGPTLLYR	1622	11	11	79	
TLPALSTGLIH	806	11	11	79	
TLWARMILMTH	2071	11	11	79	
TSCSSNVSA	2817	10	14	100	
TSCSSNVSAH	2017	11	12	86	
TSERSOPR	52	8	13	93	0.0003
TSERSOPTGR	52	10	12	86	
TSERSOPRGR	52	11	12	86	
TSLTGRDK	1050	8	12	86	
TSMITDP SH	2177	9	13	93	0.0003
TTIMAKNEVF	2589	10	11	79	
TTMRSPVF	1208	8	12	86	
TVCARQA	1597	8	11	79	
TVDFSLOTF	1466	10	12	86	
TVLDOAETA	1336	9	14	100	
TVLDOAETAGA	1336	11	12	86	
VNATLGFQA	1263	0	14	100	
VNATLGFQAY	1263	10	14	100	
VAGALVAF	1064	8	12	86	
VAGALVAFK	1064	9	12	86	0.2400
VAYQATVCA	1592	0	12	86	
VAYQATVCAR	1592	10	11	79	0.0005
VAYQATVCARA	1592	11	11	79	
VCAAILRR	1802	8	11	79	
VCAAILRRH	1802	9	11	79	
VCEKMALY	2622	8	14	100	
VOGPNYCF	505	8	13	93	
VOCHLEF	1555	8	12	86	
VCTRGVAK	1189	8	11	79	
VCTRGVAKA	1109	9	11	79	
VCMIVYHGA	1082	9	11	79	
VDFSLDPTF	1467	9	14	100	
VDILAGYGA	1854	9	11	79	
VDYPRYLWH	614	9	13	93	
VDYPRYLWHY	614	10	13	93	
VFCVOREK	2597	8	12	86	
VFOVFBKGR	2597	11	11	79	
VFPDLGVR	2614	8	11	79	

IICV A03 Motif with Binding Information

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)	A*0301
VFTGLTHIDA	1566	10	13	93	
VFTGLTHIDAH	1566	11	13	93	
VGOLGGSVF	277	9	12	86	
VGGVLAALA	1668	9	12	86	
VGGVLAALAA	1668	10	12	86	
VGGVLAALAAAY	1668	11	12	86	
VGGVLLPR	31	9	13	93	0.0003
VGGVLLPRR	31	10	13	93	
VGYLLPNR	3036	9	11	79	0.0007
VGVVCAAILR	1099	10	11	79	
VGVVCAAILRR	1099	11	11	79	
VDTLTCGF	122	9	12	86	
VDTLTCGFA	122	10	12	86	
VLAALAAAY	1671	8	12	86	
VLECYDA	1521	8	13	93	
VLECYDAGCA	1521	11	11	79	
VLDQETA	1337	8	14	100	
VLDQETAGA	1337	10	12	86	
VLDQETAGAR	1337	11	12	86	
VLEDGVNY	157	8	12	86	
VLEDGVNYA	157	9	12	86	
VLNPSVAA	1250	0	14	100	
VLTSMLTDP SH	2175	11	13	93	
VLVDILAGY	1052	9	11	79	
VLDILAGYGA	1052	11	11	79	
VLGGVLA	1668	8	12	86	
VLGGVLA	1668	9	12	86	0.0003
VLGGVLAALA	1660	11	12	86	
VLNLNPSVA	1256	9	14	100	0.0003
VLNLNPSVAA	1256	10	14	100	
VMGSSYGF	2639	8	11	79	
VMGSSYGFQY	2639	10	11	79	
VTRHADVPVR	1130	11	11	79	
VVCAAILR	1901	8	11	79	
VCAAILRR	1901	9	11	79	
VCAAILRRH	1901	10	11	79	
VGVVCAAA	1098	8	11	79	
VGVVCAAILR	1098	11	11	79	
VWGTIDR	517	8	13	93	
WAGWLLSPR	93	9	12	86	
WAGWLLSPR	1766	8	12	86	
WAGWLLSPR	76	11	12	86	
WAGWLLSPR	2073	9	12	86	
WARMILMTH	2073	10	12	86	
WARMILMTHF	2073	11	12	86	
WARMILMTHFF	2873	11	12	86	

HCV A03 Motif with Binding Information

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)	A*0301
WGPTDPRR	107	0	12	86	
WGPTDPRR	107	9	12	86	
WGPTDPRISR	107	11	12	86	
WLLSPRGR	96	9	12	86	0.0008
WMNRLIAF	1920	8	14	100	
WMNRLIAFA	1920	9	14	100	0.0003
WMNRLIAFASR	1920	11	14	100	
WMNSTGFTK	557	9	11	79	0.0530
WVLVGGVLA	1665	9	12	86	
WVLVGGVLA	1665	10	12	86	
YATGNLPGCSF	164	11	12	86	
YDAGCAWY	1526	0	11	79	
YDIIICECH	1315	10	12	86	
YGAGVAGA	1060	0	12	86	
YGAGVAGALVA	1060	11	12	86	
YGEQYSPQOR	2644	10	11	79	
YLLPRRGR	35	9	13	93	0.0054
YLVAYOATVCA	1590	11	12	86	
YSPGENR	2930	8	11	79	
YSPGENRVA	2930	10	11	79	
YSPGQVEF	2648	9	11	79	
YSTYGHFLA	1298	9	12	86	
YWGDLGSMF	276	10	12	86	
YGGVBR	637	0	14	100	
YVPESDAA	1930	0	12	86	
YVPESDAA	1939	9	12	86	
YVPESDAAR	1939	10	12	86	0.0003
567		3	12	06	

Table XVII
HCY A11 Motif With Binding Information

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)	A*1101
AACWTRGER	647	10	12	86	0.0140
AARALAHGVR	147	10	11	79	
AATLFGAY	1284	9	14	100	
AAVCTRGVAK	1187	10	11	79	
AONWTRGER	648	9	12	86	
ADGCSGGAY	1306	10	11	79	
ADVIPRR	1142	8	12	86	
ADVIPRRR	1142	9	11	79	
AFASRGNH	1925	8	14	100	
AGALVAFK	1865	8	12	86	
AGVAGALVAFK	1862	11	12	86	
AGWLLSPR	94	0	12	86	
AGWLLSPGSR	94	11	12	86	
ALSTGLIH	689	8	12	86	
ALSTGLHII	689	10	12	86	0.0027
ASQLSAPSLK	2208	10	11	79	
ASRGNHVSPTH	1928	11	12	86	
ATLGFAY	1265	0	14	100	
ATLGFAYMSK	1265	11	12	86	
ATRKTSER	48	8	11	79	0.0250
AVCTRGVAK	1180	9	11	79	
CAAILRIH	1903	8	13	93	
CGFADUMGY	128	9	13	93	
CGNTLCY	2742	0	11	79	
CGSSDLYLVIR	1130	11	12	86	
CLRQLGVPLR	2841	11	12	86	
CNCSIYFGH	304	9	11	78	
CNWTRGER	049	0	12	86	
CSSNVSVAH	2019	9	12	86	
CTGSSDLY	1128	9	11	79	0.0063
CTWAINSTGFTK	555	11	11	79	0.7500
CVOPEKGGK	2599	9	11	79	0.0005
CVOPEKGGK	2599	10	11	79	0.0008
DAHFLSOTK	1574	9	14	100	0.0005
DGCSGGAY	1307	9	11	79	
DIICDECH	1316	9	12	86	
DLGVRCCK	2617	9	13	93	0.0002
DLVLVTRH	1134	8	12	86	
DVIPRRR	1143	8	11	79	
ECTDAGCAWY	1524	10	11	79	
EGAVQWNNRI	1915	9	14	100	0.0014
ENGGNTR	2245	8	12	86	
EVFOVPEK	2596	9	12	86	0.0270
EVFOVPEKGR	2598	10	11	79	
EVFOVPEKGGK	2598	11	11	78	

HCV A11 Motif With Binding Information

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)	A*1101
FGAYMSKAH	1269	9	12	86	
FGYGAQVRI	2554	9	12	86	0.0005
FILLADAR	728	8	14	100	
FTEAMTRY	2782	8	14	100	
FTGLTHIDAH	1567	10	13	93	
GAARALAH	146	8	11	79	
GAARALAHGVR	146	11	11	79	
GAVQWNR	1916	8	14	100	
GAYMSKAH	1270	8	12	86	
GFAQMGY	120	8	13	93	
GFGAYMSK	1260	8	12	86	
GFGAYMSKAH	1260	10	12	86	
GFOYSPQOT	2645	9	11	79	
GGARALAH	145	9	11	79	
GGCSGGAY	1308	8	11	79	
GGGQVGGY	26	10	14	100	
GGQMGVY	27	9	14	100	0.0001
GGHLPFH	1382	9	14	100	
GGHLPFHCHSK	1392	11	14	100	
GGVLAALAAAY	1669	10	12	86	
GGVLLPR	32	8	13	93	
GGVLLPRR	32	9	13	93	0.0010
GYLLPNR	3037	8	11	79	
GLPVQDH	1552	8	13	83	
GLPVSAIR	1004	8	11	79	
GLSAFSLH	2821	8	11	79	
GLSAFSLHISY	2021	10	11	79	0.0005
GLTHIDAH	1568	8	13	93	
GNHVSPTH	1931	8	12	86	
GNHVSPTHY	1931	9	12	86	
GNITVESENK	2248	11	12	86	
GSSDLVLTIR	1131	10	12	86	
GSSDLVLTIRH	1131	11	12	86	
GSSYGFQY	2641	8	11	79	
GTFPINAY	2063	8	11	79	
GVAGALVAFK	1863	10	12	86	1.4000
GVQWTVVH	1081	8	11	79	
GVGYLLPNR	3035	10	11	79	0.0140
GVLAALAAAY	1670	9	12	86	0.0110
GVRAIRKTSER	45	11	11	79	
GVRAIRKTSER	2619	11	14	100	
GVRAIRKTSER	154	11	12	86	
GVVCAAILR	1900	8	11	79	
GVVCAAILR	1900	10	11	79	
GVVCAAILRRH	1900	11	11	79	

UCV A11 Motif With Binding Information

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)	A*1101
GVLLPRR	33	0	13	93	
GVLLPRGR	33	11	13	93	
HAQVPR	1141	8	11	79	
HAQVPRR	1141	9	11	79	
HAQVPRRR	1141	10	11	79	
HAPGSGK	1234	8	14	100	
HAPGSGKSTK	1234	11	13	93	
HGLSAFSLH	2920	9	11	79	
HGLSAFSLHSY	2920	11	11	79	
HGPTRLY	1624	0	11	79	
HGPTPLLYR	1624	0	11	79	
HIDAHFLSOTK	1572	11	14	100	
HLHAPGSGK	1232	10	12	86	0.0024
ILIQNVDOY	686	11	11	79	
HLFCHSK	1395	0	14	100	
HLFCHSKK	1395	9	14	100	0.0006
HLFCHSKKK	1395	10	14	100	0.0002
HMMNFSGIOY	1769	11	13	93	
HSYSPGENR	2920	10	11	79	
HTPGCVPCVR	222	10	11	79	
IAPASRGNH	1925	9	14	100	0.0012
IDNIFLSOTK	1573	10	14	100	0.0003
IFCHSKKK	1397	0	14	100	
IIIDCEH	1317	6	12	86	
INTNGSWH	415	8	11	79	
ITRVESENK	2250	9	12	86	
ITYSTYCK	1208	8	12	86	0.0079
NDVOYLY	701	0	12	86	
NFPDLGVR	2613	9	11	79	
INGGVLLPR	30	10	13	93	0.0044
INGGVLLPRR	30	11	13	93	0.0056
KDELAAR	1404	8	12	86	
KFGYGAKOVR	2553	10	12	86	
KGGRLHFOH	1391	10	11	79	
KGGRLPAR	2804	0	11	79	
KLGVPLR	2944	8	12	86	
KNEVFOVPEK	2594	11	11	79	
KSTKVPAAAY	1241	9	12	86	0.0001
KTKRNTNR	10	8	12	86	
KTKRNTNR	10	9	12	86	
KTSERSOPR	51	9	13	93	0.0100
KTSERSOPRGR	51	11	12	86	0.0640
LADGGCGGAY	1305	11	11	79	
LAQPKCK	1729	8	12	86	
LDOAETAGAR	1338	10	12	86	

HCY ΔII Motif With Binding Information

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)	A*1101
LFLLADAR	727	9	14	100	
LTFSRR	290	8	11	79	
LGFGAYMSK	1267	9	12	86	0.2900
LGFGAYMSKAH	1267	11	12	86	
LGGAARALAH	144	10	11	79	
LGVRATRK	44	8	12	86	
LGVRVCEK	2618	8	14	100	
LAFAASGNI	1924	10	14	100	
LEANLLWR	2235	9	12	86	0.0005
LIFCHSKK	1398	8	14	100	
LIFCHSKK	1398	9	14	100	0.1900
LINTGSMH	414	9	11	79	
LIVFDLGR	2612	10	11	79	0.0001
LLAPITAY	1030	8	14	100	
LLFLLADAR	726	10	14	100	
LJPRGPR	36	8	13	93	
LLSPRGR	97	8	12	86	
LSAFLHSY	2922	9	11	79	0.0002
LSNSLRFH	2479	8	12	86	
LSNSLRFH	2479	9	12	86	0.0001
LSTGLHLH	680	9	12	86	
LTCGFAJMGY	126	11	12	86	
LTSMLTDPST	2178	10	13	93	
LVAYQATVCAR	1591	11	11	79	
LVQILAGY	1053	8	11	79	
MGFSYDTR	2668	8	11	79	
MGSSYGFOY	2640	9	11	79	
MNPLAFASR	1921	10	14	100	
MNSTGFTK	550	8	11	79	
MSTNPKPOR	1	9	11	79	
MSTNPKPOR	1	10	11	79	
NOGYRRCR	2726	8	11	79	
NCSYRPGH	305	8	11	79	
NFSGIOY	1772	8	14	100	
NGVQWTVY	1080	8	11	79	
NGVQWTVYH	1080	9	11	79	
NITRVESENK	2249	10	12	86	0.0062
NVDVQVLY	700	9	12	86	0.0140
NTNRRPOOVK	14	10	11	79	0.0007
NTPLPVCCH	1549	11	13	93	
PALSTGLH	688	9	12	86	
PALSTGLHLH	688	11	12	86	
PCGSSWLR	1976	8	11	79	
PCTGSSDLY	1127	10	11	79	
PDLGVRVCEK	2616	10	13	93	

ILCV A11 Motif With Binding Information

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)	A*1101
PGCVPCVR	224	8	12	86	
PGEGAVQVMN	1913	11	13	93	
PGGGONGGVY	25	11	14	100	
PGLPVCOOH	1551	9	13	93	
PGYPWPLY	79	8	14	100	
PITYSTYCK	1295	9	11	79	
PLGGAAHALAH	143	11	11	79	
PMGFSYDTR	2687	9	11	79	
PNIRTGVR	1281	8	13	93	
PSPVWVGTDTR	514	11	13	93	
PSWDMWK	1607	8	11	79	
PTDCFRKH	507	8	12	93	
PTDPRRSR	109	9	12	86	0.0005
PTGSGSKTK	1236	9	13	93	0.0001
PTLHGPTPLLY	1621	11	11	79	0.0005
PWVGTTDR	516	9	13	93	
QAETAGAR	1340	8	12	86	
QMGVGYLLR	29	11	13	93	
QLTFSPR	289	8	12	86	
QLTFSPRR	289	9	11	79	0.0330
QLSAPSLK	2210	8	11	79	
QNVQVQY	699	8	11	79	
QNVQVQYLY	699	10	11	78	
RAAVCTRGVAK	1100	11	11	79	
RAVAHGVR	149	8	14	100	
RATKNTSER	47	9	11	78	
RGNHVSPTH	1830	9	12	86	0.0001
RGNHVSPTH	1830	10	12	86	0.0001
RGPLGVR	40	8	13	93	
RGPLGVRATR	40	11	11	79	
RGRROPPIK	59	9	13	93	0.0017
RGSLSRPR	1154	8	12	86	
RLGVRATR	43	8	11	79	0.0290
RLGVRATRK	43	9	11	79	
RLHQLSAFLH	2918	11	11	79	
RLIAFASR	1923	8	14	100	
RLIAFASRGNH	1923	11	14	100	
RLMFPLGVR	2611	11	11	79	
RLAPITAY	1029	9	12	86	0.0270
RMVVGVEH	635	9	14	100	
RMVVGVEH	635	10	14	100	0.0200
RNTNRPODMK	55	11	11	79	
RSCPRGR	55	0	13	93	
RVCEKMALY	2621	9	14	100	0.5000
RVLEDGWTY	158	9	12	86	0.0068

HCV Δ11 Motif With Binding Information

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)	A*1101
SAFSLHSY	2923	8	11	79	
SASOLSAPSLK	2207	11	11	79	
SCSSNWSVAH	2818	10	12	86	
SDLYLVTR	1133	8	12	86	
SDLYLVTRH	1133	9	12	86	
SGKSTKVPAA	1239	11	12	86	
SMLTDPH	2178	8	14	100	
SNLSLPHH	2400	8	12	86	
SSDLYLVTR	1132	9	12	86	0.0044
SSDLYLVTRH	1132	10	12	88	0.0013
SSNWSVAH	2020	8	12	86	
STGLIHLH	691	8	12	86	
STKVPAA	1242	8	12	86	
STNPKPOR	2	9	11	79	
STNPKPORK	2	11	11	79	
STNPKPORKTK	2	11	14	100	
SVAATLGFAY	1262	10	13	93	
TCGFAQLMGY	127	8	11	79	
TCSSDLY	1129	8	12	86	
TDPIHRSR	110	9	11	79	0.0001
TGEIPFYK	1375	9	13	93	
TGLTHDAH	1588	9	13	93	
TGSGKSTK	1237	8	12	86	
TLGFGAYMSK	1268	10	12	86	0.0610
TLHGPTLLY	1622	10	11	78	0.0007
TLHGPTLLYR	1622	11	11	79	
TLPALSTGLH	800	11	11	79	
TLWARMILMTH	2971	11	11	70	
TNPKPORK	3	8	11	79	
TNPKPORKTK	3	10	11	78	
TNPKPORKTKR	3	11	11	79	
TNPKPOOVK	15	9	11	78	
TSCSSNWSVAH	2917	11	12	86	
TSERSOFH	52	8	13	93	
TSERSOPTGR	52	10	12	86	0.0001
TSERSOPTGRH	52	11	12	86	
TSLTGRDK	1050	8	12	86	
TSMLTDFSH	2177	9	13	93	0.0001
VAATLGFAY	1263	10	14	100	
VAGALVAFK	1864	9	12	86	0.8900
VAYDATVCAR	1592	10	11	79	0.0038
VCAAILRR	1802	8	11	79	
VCAAILRRH	1902	9	11	79	
VCEKMLY	2622	8	14	100	
VCTRGVAK	1189	8	11	79	

HCV A11 Motif With Binding Information

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)	A*1101
VDYPYRLWH	614	9	13	93	
VDYPYRLWHY	614	10	13	93	
VFCVPEK	2587	8	12	86	
VFCVPEKGR	2587	11	11	79	
VFDLGVH	2614	8	11	78	
VFTGLTHDAH	1566	11	13	93	
VGGVLAALAA	1668	11	12	86	
VGGVLAALAA	31	9	13	93	0.0018
VGGVYLPFR	31	10	13	93	
VGNLLPFR	3036	9	11	79	0.0100
VGVVCAILR	1809	10	11	79	
VGVVCAILRR	1899	11	11	79	
VLAALAA	1871	8	12	86	
VLDQAETAGAR	1337	11	12	86	
VLEDGWY	157	0	12	86	
VLTSMLTDFSH	2175	11	13	93	
VLVDILAGY	1852	9	11	79	
VMSSTYGFQY	2638	10	11	79	
VTRHADVIPVR	1138	11	11	79	
VVCANLR	1901	8	11	79	
VVCANLRR	1901	9	11	79	
VVCAILRRH	1801	10	11	79	
VVGWCAILR	1008	11	11	79	
VVGTTDR	517	0	13	93	
WAGWLLSPH	93	0	12	86	
WAGPGYFWPL	78	11	12	86	
WARMILMTH	2873	0	12	86	
WGFTDPIR	107	8	12	86	
WGFTDPIRR	107	9	12	86	
WGFTDPIRRSR	107	11	12	86	
WLLSPRGR	96	9	12	86	0.0005
WNNRLIAFASR	1920	11	14	100	
WNNSTGFTK	557	9	11	79	0.0810
WNRSGIY	1771	9	14	100	
YDAGCAWY	1526	8	11	79	
YDIICDECH	1315	10	12	86	
YGFQYSPQGR	2844	10	11	79	
YLLPRGPR	35	9	13	93	0.0005
YSPGENR	2930	8	11	79	
YGGVBR	637	8	14	100	
YVPESDAAR	1938	10	12	86	0.0001
311		3			

Table XVIII
ICY A24 Motif With Binding Information

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)	A*2401
AWDMIMNW	319	0	12	86	
AYAAQYKVL	1248	10	11	79	0.0009
AYYRGLDVS	1421	11	14	100	
CYDAGCAW	1525	0	11	79	
CYDAGCAWTEL	1525	11	11	79	
DFSLDPTF	1468	0	14	100	
DFSLDPTFI	1468	10	14	100	
FWAKHMMWF	1765	9	12	86	6.9000
FWAKHMMWFI	1765	10	12	86	
GFADLMGYI	129	9	13	93	
GFADLMGYIPL	129	11	11	79	
GFSYDTRCF	2669	0	11	79	
GWRLIAP	1027	0	11	79	
QYGAGVACAL	1059	10	12	86	0.0003
GYPLVGAPL	135	10	11	79	0.0057
GYRPRASGVL	2728	11	12	86	
HMMNFSGI	1769	9	13	93	
IFLLALLSCL	176	10	12	86	
IMANVEF	2591	8	12	86	
KFPGGGQ	23	8	13	93	
LNVLGGW	1813	8	12	86	
LWARMILMTHF	2872	11	12	86	
LWROEMGNI	2241	10	12	86	
LYLVTRHADV	1135	11	11	79	
MWNFSGI	1770	0	14	100	
MWNFSGIYI	1770	11	14	100	
MYGGVBEPL	636	10	13	93	0.0270
NFISGIVL	1772	9	14	100	0.0170
PMGFSYDTRCF	2667	11	11	79	
QFKKALGL	1732	9	12	86	
QFKKALGLL	1732	10	12	86	
QWMNRLAF	1919	9	14	100	
QYLAGLSTL	1778	9	14	100	
QYSPQORVEF	2647	10	11	79	0.0480
QYSPQORVEFL	2647	11	11	79	0.0180
FINAWDMIMNW	317	10	12	86	
FMILMTHF	2875	8	12	86	
FMILMTHFF	2875	9	12	86	
FMVGGVBEPL	835	11	13	93	
SFSIFLLAL	173	9	14	100	0.0041
SFSIFLLALL	173	10	14	100	
SMILDFPSHI	2178	9	14	100	
SWDMMWCOL	1608	9	11	79	
SYUGSSGGPL	1164	11	12	86	
TWMNSTGF	556	8	11	79	

IICV Δ24 Motif With Binding Information

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)	A*2401
TWLVGGVL	1664	9	12	86	
TYSTYGKF	1297	8	13	93	
TYSTYGKFL	1297	9	12	86	0.0230
VFTGLTH	1566	8	13	93	
VACSSYGF	2638	8	11	79	
VYLLPIRGPRLL	34	11	13	93	0.0016
WMNRLLAF	1920	8	14	100	
YYRGLDYSVI	1422	10	14	100	
53		2			

Table XIX a
HCV DR-Super Motif

Core Sequence	Core Freq.	Core Consistency (%)	Exemplary Sequence	Position in HCV Poly-protein	Exemplary Sequence Frequency	Exemplary Sequence Consistency (%)
FOATMSWNI	12	80	TILGENTASKNIQVQ	1286	5	36
FGCTWAST	12	86	GNMFGCTMASTGFT	550	11	79
FKOKALGIL	12	86	AECHQKIALDLOIA	1730	12	88
FLUALLSCL	12	86	FSFLLALSCLTYP	174	6	43
FDLGVNRC	11	79	LNPFQGVNRCENM	2612	11	79
FOVAILHAP	12	86	POTFOVAILHAPLOS	1225	8	42
FRACVITG	12	86	YORFRACVITGVAK	1182	7	60
FSFLALL	14	100	CCSFSLALLSQL	171	12	86
FSLDPFTI	14	100	TVDFSLDPFTIETI	1466	11	79
FTEAMTRY	14	100	LVNTEAMTRYGAPP	2769	7	50
FTSPAAQD	13	93	VYCFPSPAQDITD	509	13	93
FTLLPALST	11	79	PCSTFLPALSTIGLI	681	9	64
FYAKIWAH	12	80	LEVYWAHAKWNTSQ	1702	3	21
IDNIFLSOT	14	100	LTIDNIFLSQIKQA	1570	7	50
IDCHCVIQ	12	86	DSYDCHCVIQVQD	1454	12	86
IDILICQFA	12	88	OKWIDILICGFADLM	120	12	88
IEAMLIWQD	12	86	AUXIEAMLIWQDEWQ	2233	7	50
IFLLALLSC	14	100	SFSIFLLALLSQLIV	173	6	43
ILGGWAAQ	12	88	LFNIGGWAAQALP	1813	8	57
ILGHTYLD	12	86	STILGHTYLDQAE	1328	8	57
ILPHAGPD	11	79	CAWILPHAGPDGA	1903	11	79
ILSPQALVV	13	93	LPAILSPQALVQWV	1888	11	79
INVTITGTC	12	86	TFNVTITGTCPTPS	2084	8	57
IFLVQAPLG	11	79	ADYFLVQAPLGQMA	134	10	71
ITITVESBK	12	86	GGNITITVESBKVR	2247	10	71
ITSCSSNWS	14	100	LEUITSCSSNWSVLI	2013	10	71
NFRLQVNI	11	79	AILNFRLQVNIQCE	2610	11	79
LAALAAVCL	12	86	GVVLAALAAVCLTQ	1669	8	57
LVKQCCQD	11	79	GVVLAQCCQDQVQ	1202	10	71
LVGLSTLPG	14	100	GVVLAQGLSTLPHVA	1777	14	100
LVQVQAGVA	11	79	VQLVQVQAGVQVQAL	1834	10	71
LVATIPRGS	12	86	LVVLAATIPRGSVTV	1348	8	64
LDPTFRET	12	86	DFSLDPTFRETIV	1468	5	38
LDQAGTAA	12	86	QVLDQAGTAAQRLV	1335	12	86
LEUISLSS	13	93	EYDLEUISLSSNWS	2810	13	93
LEVVTSTWV	12	86	SADLEVVTSTWVLQ	1653	11	79
LFLLADAR	14	100	VVLELLELLADARVCS	724	4	29
LGQWVAQDL	12	86	FNLGQWVAQDLAPP	1814	8	57
LGQTVLDD	13	93	TILGQTVLDDQET	1329	8	57
LVYATIRKT	12	86	QPLVYATIRKTISEI	41	10	71
LVVRCENM	14	100	FDLVVRCENMVALY	2615	11	79
LVGLSVFSL	11	79	ISFVLGLSVFSLVSY	2916	6	43
LVQPTIRLY	11	79	NTVLQPTIRLYLQ	1620	11	79
LVQNVQVQ	12	86	LVHQLQNVQVQVLY	694	10	71
LVSTSPQEI	11	79	AFSLVSTSPQENTV	2924	11	79
LVAFSPGN	14	100	MAFLVAFSPGNNS	1921	12	86
LVKANLWR	12	86	DAFLKANLWRQDEM	2232	7	50
LVCSBK	14	100	QPLVFLVCSBKQDE	1393	14	100
LVSCSSW	14	100	QRELVSCSSWVA	2812	13	93
LVALLSCLT	12	86	SFVLLALLSCLTNPA	176	5	36
LVFLLLADA	14	100	TVVLLFLLLADARVC	723	5	36
LVFNILQWV	12	86	QNTLVFNILQWVAA	1009	4	29
LVADARVC	13	93	LVLLLVADARVCACL	726	8	64
LVNLSPO	13	93	LVNLLVNLSPQALV	1684	10	71

HCV DR-Super Motif

Core Sequence	Core Freq.	Core Consensus (%)	Exemplary Sequence	Position in HCV Poly-protein	Exemplary Sequence Frequency	Exemplary Sequence Conservancy (%)
UMGTFVQ	11	78	FQIMGTFVQ	130	11	79
LNPSVAATL	14	100	VLVNPSVAATLQ	1256	14	100
LPALSPGA	13	93	VALLPALSPLQV	1865	11	79
LPALSTOU	12	86	FTLPALSTQVH	684	11	79
UPRTPTG	13	93	VYLPRTPTQVIA	34	13	93
LDLAVAE	11	79	HNELRLAVNEPV	966	4	29
UNKQVPL	12	86	ASQUNKQVPLN	2939	7	50
LSA-SVSY	11	79	UGLSA-SVSYSG	2919	11	79
LSAPSLAT	11	79	ASQSLAPSLATCT	2208	7	50
LSNSLPH	12	86	INALNSLPHHAY	2476	4	29
LSPGALVQ	13	93	PALSPGALVQVC	1889	11	79
LSPLSTT	11	79	NSLSPLSTTENG	664	7	50
LSYTSST	11	79	QWLSYTSSTSWP	95	11	79
LSQLRLH	12	86	LPALSQRLHQN	687	10	71
LTCGFQAL	12	86	RLTCGFQALQNY	123	12	88
LTHDAEL	13	93	FTLTHDAELSY	1567	13	93
LTSMLTDS	13	93	VALLTSMLTDSH	2173	9	64
LVAYQATVC	12	86	FPYLVAQATVCADA	1508	9	64
LVGLAGTG	11	79	GKVLGLAGTGAV	1850	8	64
LVGGVLAAL	12	86	TWYLVGGVLAALAY	1664	12	88
LVNPSVAA	14	100	VKVLNPSVAAILQ	1254	14	100
LVNLPAIL	11	79	TEQLVNLPAILSPQ	1881	10	71
LVTRIDNH	11	79	GLYLVTRIDNHVPH	1134	11	79
LVYQVCA	11	79	PKLVYQVCAQEN	1094	11	79
LVVLATATP	12	86	QAILVVLATATPDS	1345	11	79
LWATMLMT	12	86	APLWATMLMTIEF	2859	11	79
LWDEMQN	12	86	ANLWDEMQNHIN	2238	11	79
LYTLOAVCN	11	79	ITLYTLOAVCNVET	1627	9	64
MAHNEVFCV	12	86	ITMAHNEVFCVDE	2509	8	64
MAWQNNNNW	12	86	QFMAWQNNNNWSEPT	315	12	86
MOQNITVE	12	86	FOEQMOQNITVESEN	2243	12	86
MYELVDA	11	79	ADLYMYELVDAILO	131	11	79
MLTDSHIT	14	100	LTSMMLTDSHITAEI	2178	8	57
MYRLVAFS	14	100	YOMMYRLVAFSGEN	1918	14	100
MYRYSAPD	14	100	TEAMMYRYSAPDGP	2793	10	71
MYRFSQD	14	100	AKMYRFSQDQYLA	1767	12	86
MYQGVCHR	14	100	KYRMYQGVCHFLNA	633	5	35
VAGALVAFK	12	86	GADVAGALVAFKNS	1861	7	50
VARLHPTG	12	86	TQVVARLHPTGSK	1227	6	43
VATDALMTG	12	86	VYVATDALMTGYTG	1437	6	43
VAYQATVCA	12	86	PYLVAQATVCADQ	1589	11	79
VCAALRIRI	11	79	VQVCAALRIRNGP	1899	10	71
VCEKVALYD	14	100	QVTVCEKVALYDVVS	2619	11	79
VQDDLEFW	12	86	GLPVQDDLEFWESV	1552	6	43
VCTRQVAA	11	79	DAVCTRQVAVKQDF	1186	11	79
VFOVDFEKO	12	86	MAVFOVDFEKGK	2594	10	71
VFTDNSSPP	11	79	TSVFTDNSSPPAP	1211	10	71
VFTDLTHD	13	93	WESVFTDLTHDAF	1563	6	43
VGGVLAALA	12	86	WYLVGGVLAALAYC	1655	12	86
VGVVALLR	13	93	GVNVGVVALLRTECP	28	13	93
VGSQVCEP	12	86	QVNVGSQVCEPDP	2158	6	43
VGVVCAVL	11	79	ALYGVVCAVLIRH	1898	11	79
VQCNVCVT	12	86	FDSVQCNVCVTDTV	1453	12	86
VDTLTCGF	12	86	LQNVDTLTCGFADL	119	11	79

Core Sequence	Core Freq.	Core Consistency (%)	Exemplary Sequence	Prdlin In HCV Prty-protein	Exemplary Sequence Frequency	Exemplary Sequence Consistency (%)
VLALAAATC	12	86	VGVLAAALAAVLT	1668	8	57
VLATATPRG	13	93	RLVLATATPRGSVT	1347	8	64
VLEGGUNTA	12	86	GVRVLEGGUNYATGN	154	12	86
VLEGGUNTA	14	100	KVLVLEGGUNYATGN	1255	14	100
VLEGGUNTA	13	93	DYVLEGGUNYATGN	2172	9	64
VLEGGUNTA	11	79	ASVLEGGUNYATGN	2734	10	71
VLEGGUNTA	11	79	ASVLEGGUNYATGN	1849	10	71
VLEGGUNTA	11	79	ASVLEGGUNYATGN	1653	12	86
VLEGGUNTA	12	86	STVLEGGUNYATGN	1253	14	100
VLEGGUNTA	14	100	STVLEGGUNYATGN	1882	11	79
VLEGGUNTA	12	86	EDVLEGGUNYATGN	1937	7	50
VLEGGUNTA	12	86	EDVLEGGUNYATGN	1658	12	86
VLEGGUNTA	12	86	EDVLEGGUNYATGN	1436	6	43
VLEGGUNTA	11	79	DVLEGGUNYATGN	1898	10	71
VLEGGUNTA	11	79	DVLEGGUNYATGN	1895	11	79
VLEGGUNTA	11	79	DVLEGGUNYATGN	1346	9	84
VLEGGUNTA	12	86	ARVLEGGUNYATGN	506	13	93
VLEGGUNTA	13	93	CGVLEGGUNYATGN	90	5	35
VLEGGUNTA	12	86	CGVLEGGUNYATGN	2870	11	79
VLEGGUNTA	12	86	PTVLEGGUNYATGN	988	6	43
VLEGGUNTA	12	86	ITVLEGGUNYATGN	104	10	71
VLEGGUNTA	12	86	ITVLEGGUNYATGN	1917	14	100
VLEGGUNTA	14	100	ACVLEGGUNYATGN	1025	4	28
VLEGGUNTA	11	79	SKVLEGGUNYATGN	2456	9	64
VLEGGUNTA	11	79	SYTVLEGGUNYATGN	1529	5	36
VLEGGUNTA	12	86	GCVLEGGUNYATGN	161	11	79
VLEGGUNTA	12	86	GCVLEGGUNYATGN	507	13	93
VLEGGUNTA	12	86	GCVLEGGUNYATGN	1523	10	71
VLEGGUNTA	11	79	GCVLEGGUNYATGN	1312	10	71
VLEGGUNTA	12	86	GCVLEGGUNYATGN	2808	79	79
VLEGGUNTA	13	93	GCVLEGGUNYATGN	1857	11	79
VLEGGUNTA	12	86	GCVLEGGUNYATGN	2641	10	71
VLEGGUNTA	11	79	GCVLEGGUNYATGN	1298	10	71
VLEGGUNTA	14	100	GCVLEGGUNYATGN	1251	11	79
VLEGGUNTA	14	100	GCVLEGGUNYATGN	1776	14	100
VLEGGUNTA	12	86	GCVLEGGUNYATGN	1162	6	43
VLEGGUNTA	12	86	GCVLEGGUNYATGN	2633	9	64
VLEGGUNTA	11	79	GCVLEGGUNYATGN	1591	11	79
VLEGGUNTA	13	93	GCVLEGGUNYATGN	1420	7	50
VLEGGUNTA	14	100	GCVLEGGUNYATGN	1628	9	64
VLEGGUNTA	11	79	GCVLEGGUNYATGN	2726	10	71
VLEGGUNTA	13	93	GCVLEGGUNYATGN	2802	5	43
VLEGGUNTA	11	79	GCVLEGGUNYATGN	2927	8	57
VLEGGUNTA	12	86	GCVLEGGUNYATGN	273	8	57
VLEGGUNTA	11	79	GCVLEGGUNYATGN	3036		

UCY DR Super Motif With Binding Data

Core Sequence	Exemplary Sequence	DR1	DR2=2.1	DR3	DR4=4	DR4=5	DR5=11	DR5=12	DR5=19	DR6=2	DR7	DR8	DR1=1.1
UNPSVAATL	VLVNPVAATLQFG	1.4000	0.0004		2.1000	0.0005	0.0140		0.3100	0.0012	1.5000		3.2000
UPALSPGA	VLLPALSPGALVY	4.3000	0.0016		0.0071		0.0130		0.0002		0.4000		0.0310
LPALSTGL	FTLLPALSTGLRLH	0.0140	0.0360		0.0014		0.0120		0.0001		-0.0003		0.0032
UPTEQTLL	VYLPTEQTEPLOGVA												
LPRLAVAVE	PNQLRLAVAVEPWW												
UPKLVPL	ASCLPKLVPLTWW	1.0000	0.0920	0.0051	0.0080	0.4900	0.0330	1.9000	0.0014	0.0730	0.0290		0.0087
LSAFSLHY	UGLSAFSLHYSPG	1.6000			0.0095						0.0070		
LSAPSLKAT	ASOLSAPSLKATCTT	0.0150			0.0056						0.0008		
LSNLSLPHH	INALSNLSLPHHNAV												
LSPOLWQ	PALSPGALVQWVC												
LSPLLSIT	RSELSPLLSITTEWQ												
LSPLSPTS	GMALSPHSQSPSWGP												
LSLGLLUI	LPALSTGLILION												
LTCGFALIA	IDILTCGFALIAQVI	0.0017			0.0024								
LTHIDALF	FIGLTHIDALFISOT	0.7600	0.1300		0.0005	0.0020	0.0020		0.0002	0.0600	-0.0003		0.0056
LTSALTOPS	VAULTSALTOPSIBT										0.1400		
LVAYDATIC	FPYLVAAYDATICARA												
LVOLAGYO	GKMLVOLAGYOGVQV	0.7700	-0.0003		0.0015		0.0008		0.0001		0.0570		0.0058
LVGGLAAL	FWVLVGGLAALAAV												
LVNPSVAA	VKVLNPSVAAITLG												
LVNLPAL	TECLVNLPALSPQ												
LVTRHAYI	DLVTRHAYIDNPPH												
LVGVVCAA	PQNLVGVVCAANLI	0.0081	0.0220		0.0018				0.0005		0.0810		0.0820
LVVLATATP	QAILLVVLATATPLOS	0.0300	0.0009		0.0000		0.0076		0.0004		0.0440		0.0067
LWNCMLAT	APLWNCMLATMHFF				0.0000		0.0094						
LWTCMCKN	ALLWTCMCKNTIN	0.7000											
LYRLGAVON	LYLYRLGAVONEVT				0.0018						0.0022		
MAKNEVCY	TRMAKNEVCYQTE	0.0014			0.0016						0.0025		
MAVJAMANN	GMMAVJAMANNHSTI	0.0200	0.0044		0.1000		0.0079		0.0000		0.0017		0.0230
MACTUTINE	PQCLMACTUTINEEN	0.0001			-0.0003						-0.0002		
MAOTELVIA	ALLMAOTELVIAQPIQ	0.0006			0.0060						0.0018		
MAOTPSHT	LTSMAOTPSHTAET	0.0004			0.0740						-0.0003		
MAVILVAFS	WQMAVILVAFSTON												
MIRTSAPFG	TEAMIRTSAPFGZPP	1.5000	0.0150		0.0040	0.0020	0.0076		0.0004	0.0100	0.2300		0.2700
MYNFSQO	AGMYNFSQOYLA												
MYGQVGR	KVRYGQVGRNFIUNA												
VAGALVAFK	GAGVAGALVAFKVAS												
VAVLHAPTO	TECVAVLHAPTOGSK												
VATDALMITO	VYVATDALMITOTIG	0.0048	0.0047	1.1000			0.0008		0.0029	0.0029	0.0400		
VAYDATIC	FPYLVAAYDATICARAQ												
VCAALURRH	VGVVCAALURRHNGP												
VCEKALYD	GVVCEKALYDQVS	0.0022		0.0063	0.0012						-0.0002		
VCCZLLEFW	GLPVCCZLLEFWESV	0.0100			0.0077						0.0024		
VCTRGVWA	RLPVCTRGVWAKQDE												
VCECHERK	KQVCECHERKQGRK												
VFTDQNSPP	RSVFTDQNSPPNPP												
VFTGLTHD	WESVFTGLTHDAIE												
VGVVLAALA	WLVGVVLAALAAVYC	0.0310			0.0068						0.0005		
VGVVLLPR	GVVGVVLLPRPSP												
VGSQZCEP	QTLVGSQZCEPEPD												
VGVVCAAL	ALVGVVCAALIRRH												
VDCNCTVT	FDSVDCNCTVTQTY												
VQILTCQF	LQVQILTCQFADL												
VLAALAAYC	VQVLAALAAYCLIT												
VLATATPPQ	RLVLATATPPQSVT	0.0015			0.0096						0.0019		

HCV DR Super Motif With Binding Data

Core Sequence	Exemplary Sequence	DR1	DR2-w2.1	DR2-w2.2	DR3	DR4-w4	DR4-w5	DR5-w1.1	DR5-w1.2	DR6-w1.9	DR6-w2	DR7	DR9	DR-w3
MEQVNYA	DMVLEDMVNTATN	0.0007				0.0006						-0.0002		
VLPNSVAAT	KVLVLPNSVAATDF													
VLTSLTDP	DVAVLTSLTDPSTH													
MLTSCGNT	ASQMLTSCGNTLC													
VLVDLAGY	LQKVLVDLAGYQAG													
VLGGVCAA	STWVLGGVCAALAA													
VVLNPSVA	GKVLNPSVAATIL	1.1000	0.0260	0.0004	0.0980	9.6000	0.0570	0.1400	0.0320	0.6900	0.1700	0.2600	1.4000	
VHLLPAIS	EDLVHLLPAISQDA	0.3700				0.0110						0.0015		
VPESDAAR	THVPESDAARVTO													
VLSWLVQ	LEWLSWLVQGVLT	0.0120	0.0078	-0.0003		0.0280		0.0008		0.0046		0.1600	0.0120	
VVATDALMT	DNVAVATDALMTOT	0.0110	0.0110	-0.0003		0.0180	0.0072	-0.0004		0.0140	-0.0003	0.0910	-0.0025	
VWCAALTR	VWGWCAALTRWQ													
VVLATATPP	ANLVVLATATPPQSV	0.0170				0.0067						0.0043		
VVCFTPSV	GGPVVCFTPSVWQV	0.2700	0.0025	-0.0003		0.2600	0.4000	0.0005		-0.0001	0.0071	0.2700	0.4300	
WAGHLLSPH	GGHWHALLSPHSPH													
WATMLMIII	PILWATMLMIIFFS	0.0064				0.0200						0.0190		
WQADTAACO	HTWQADTAACQJH													
WQPTDFRR	FFSWQPTDFRRSTN													
WNNRLAFA	AVQWNNRLAFASTQ	2.2000				0.0035								
WRLAPTA	SKGWRLAPTAQAG	14.0000	0.0730	0.8600	-0.0006	2.1000	0.2500	4.2000	0.0290	-0.0001	0.9000	0.0205	0.0630	
WTCALTPC	SYHWTCALTPCAE	0.0260	0.0007	0.0015		0.0680	0.0220	0.0031		-0.0001	0.0130	0.0260	0.0750	
WTELTPAET	GCWWTELTPAETTVR													
YATGNURGC	GNWYATGNURGCFS	0.0011				0.0130						-0.0003		
YCFTPSPW	GNWYCFTPSPWMT													
YDAGCAWYE	CECYDAGCAWYELIP													
YKRDCEC	GGAYKRDCECHST													
YDLUTSC	CFEYDLUTSCSSN	0.0003				0.0004						-0.0002		
YQAGVAGAL	LAQYQAGVAGALVAF	0.0410				-0.0003						0.0008		
YQCTGSDO	QSSYQCTGSDOQNE	0.4500	0.0001	0.0390	0.0007	0.1200	0.0510	0.0010	0.0003	0.1800	0.0007	0.1500	1.1000	
YQPLUADGI	YSTQYQPLUADGECST													
YKVLVLPNS	AGQYKVLVLPNSVAA	0.8400	0.0140	0.0004	0.0045	0.3000	0.1700	0.2700	0.0370	0.5900	0.2800	0.0300	0.2000	
YKGLSLUP	GGYKGLSLUPQAP													
YKSSQCP	PVYKSSQCPALIC													
YLTROPTP	PVYLTROPTTPVART													
YQATVCABA	LVYQATVCABARQAP													
YRDLQSH	VAYRDLQSHVPISTIS													
YRDLQVONE	PVYRDLQVONEVIL													
YRDLQVASSY	NCQYRDLQVASSYVLT													
YSKETLUP	QACTYSKETLUPQDI													
YSKEDIN	USYSKEDINIVASC													
YKGLQSSV	SWAYKGLQSSVFLV				-0.0017									
YQVLLPNT														

HICV DR Super Motif With Binding Data

Core Sequence	Exemplary Sequence	DT1	DT2+2.1	DT2+2.2	DT3	DT4+4	DT4+15	DT5+11	DT5+12	DRB+2	DR7	DR9
VLEDVNYA	DVTVLEDVNYATON	0.0007									-0.0002	
VLPNSVAAT	KVLVLPNSVAATLOF					0.0006						
VLTSMLTDP	DVAVLTSMLTDPFII											
VLTSQGH	ASQVLTSQGHRLIC											
VLDLAGY	LGKVLVLDLAGYDAG											
VLVGVLA	STWVLVGVVLAALAA											
VVLVNPVA	QYKVLVNPVSAATL	1.1000	0.0260	0.0004	0.0900	9.6000	0.0670	0.1400	0.0520	0.1700	0.2800	1.4000
VNLLPALS	EDLVNLLPALSFGA	0.3700				0.0110					0.0015	
VPESDAAR	THVPESDAARVTO											
VTSTWLVG	LEVVTSTWLVGGVVL	0.0120	0.0078	-0.0003		0.0200		0.0008	0.0046		0.1800	0.0120
VVATDALMT	DWVWATDALMTGYT	0.0110	0.0110	-0.0003		0.0180	0.0072	-0.0004	0.0140	-0.0003	0.0810	-0.0025
WCAALRR	WVGWCAALRRIRVG											
WGVVCAAI	QALWGVVCAALRII	0.0170				0.0067					0.0043	
VLATATPP	AILVLATATPPQSV											
WCFTPSY	QSPYWCFTPSPWVG	0.2700	0.0025	-0.0003		0.2600	0.4000	0.0005	-0.0001	0.0011	0.2700	0.4300
WAGWLLSPR	GGWAGWLLSPRQFI											
WAVBLMIH	PIWAVBLMIHFFS	0.0064				0.0200					0.0190	
WQADTAAG	ITWQADTAAGQDI											
WQPIDPRH	FFSWQPIDPRHRSN											
WVNLVJFA	AVQWVNLVJFASQI	2.2000				0.0035					0.0205	
WRLLAITA	SKQWRLLAITAYAQ	14.0000	0.0730	0.0006	-0.0006	2.1000	0.2500	4.2000	0.0290	0.9000	0.0260	0.0630
WTDALTPC	SYTWTDALTPCAAE	0.0260	0.0007	0.0015		0.0680	0.0220	0.0031	-0.0001	0.0130	0.4900	0.0750
WYELTPAET	GCWYELTPAETTVR											
YATGALPQC	QVATGALPQCSFS	0.0011				0.0130					-0.0003	
YCFTPSPW	QVATGALPQCSFS											
YDAGCAWE	QVATGALPQCSFS											
YHICDEC	GCYHICDECQJST											
YHLEITSC	CFYHLEITSCSNH	0.0003										
YHAGVAGAL	LAHYHAGVAGALVNF	0.0410				-0.0003					-0.0002	
YHOFSTGO	USSYHOFSTGOHME	0.4600	0.0001	0.0300	0.0007	0.1200	0.0510	0.0010	0.0003	0.0007	0.0008	1.1000
YHGLADGG	YSYHGLADGGCSO										0.1800	
YKVLVLPFS	AQYKVLVLPFSVAA	0.8400	0.0140	0.0004	0.0045	6.3000	0.1700	0.2700	0.0370	0.2800	0.0300	0.2000
YUAGLSTLP	QDYUAGLSTLPNP											
YUGSSQSP	PVYUGSSQSPRLC											
YLTROFTTP	RYVYLTROFTTPLAR											
YQATVCAAP	LYVQATVCAAPADAP											
YRGLDYSVI	VAYYRGLDYSVPTS											
YRGLANONE	PLLYRGLANONEVIL											
YRFRAGSN	NOYRFRAGSNVLT											
YSIEPLDUP	QACYSIEPLDUPQI											
YSFQENIV	USYSFQENIVASC											
YQGLCOSV	SAMYQGLCOSVFLV											
YQTLVNAI					-0.0017							

Table XXb HCY DR 3A Motif With Binding Information

Core Sequence	Exemplary Sequence	DT3	DT1	DT2w201	DT2w202	DR4w4	DR5w11	DR5w12	DR6w19	DT7	DR8w2	DT9	DRw53
FLADGGCG	YGRFLADGGCGGAY												
FSLDPTFI	TYDFSLDPTFTIETI	-0.0017	0.0001			0.1600				0.0005			
LEGERGPD	MPLEGERGPDQLSD	-0.0017											
LPCEPEOV	GSOLPCEPEPDVAL		0.0200	0.0015	0.0044	0.1600	0.0079	0.0080		0.0017		0.0230	
MAVDNMMW	GHRAVDNMMARNSPT		0.0004			0.0740				-0.0003			
MLTOPSHIT	LTSALTOPSHITAET												
MSADLEWT	MACMSADLEWVTSTW												
VATDALMTG	VVVVATDALMTGYTG	1.1000	0.0048	0.0047	0.0014		0.0006	0.0029		0.0400	0.0029		
VCOOHLEFW	GLPVCOOHLEFWESV	0.0063											
VFPDLGVTV	FLMFVFPDLGVTVCEK												
VFTDSSPP	RSPVFTDSSPPAVP												
VLCGYDAG	DSSVLCGYDAGCAW	-0.0017	0.0007			0.0006				-0.0002			
VLEDGVNYA	GVFMLEDGVNYATGN												
VLVDLAGY	LGRVLVDLAGYAG												
VQFEGGRK	VFCVQFEGGRKPPAN												
YDLLEITSC	OPEYDLLEITSCSSN		0.0003			0.0004				-0.0002			
YSIEPLDLP	GACTYSIEPLDLPOR												
YWGKQGSV	SWMYWGKQGSVFLV	-0.0017											
YVPESDAAA	PTHVYVPESDAAARVT	0.0220											

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Table XXc HCV 3B Motif

Core Sequence	Core Freq	Core Consistency (%)	Exemplary Sequence	Position in HCV Poly-protein	Exemplary Sequence Frequency	Exemplary Sequence Consistency (%)
FDISWQD	14	100	11HCHSWQDELA	1395	14	100
FSIDRFQD	11	79	PAQSTQIRCRSTV	2667	11	79
LAGQFQKA	12	86	GMGLASQKQKALGL	1728	8	57
LKPTLQPT	11	79	LKPTLQKPTPL	1816	10	71
VRATKQSE	11	79	RLQVTRKTSFQ	43	10	71
YLVRHADV	12	86	SQLYLYTNADVFP	1133	11	79
ASTNPKQF	11	79		1		

Table XXd HCV 3B Motif Binding Data

Core Sequence	Exemplary Sequence	DT1	DR2w231	DR2w232	DR3	DR4w4	DR4w15	DR5w11	DR5w12	DR6w16	DR8w2	DR7	DR9	DRw53
FLSHWCD	HLFCHSWKDELA													
PSYDRCF	PMGFSTDTKQSTV													
LAEPKKA	GMQLAEQFKKALG				0.0180									
LNFTLHPT	URLKPTLHGPTLL													
VRATKSE	RLGMATKTSERSO													
YLVRADY	SDLVLRADVPIV				0.0022									
MTNKKOR														

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TABLE XXI. Population coverage with combined HLA Supertypes

<u>HLA-SUPERTYPES</u>	PHENOTYPIC FREQUENCY					
	Caucasian	North American Black	Japanese	Chinese	Hispanic	Average
<u>a. Individual Supertypes</u>						
A2	45.8	39.0	42.4	45.9	43.0	43.2
A3	37.5	42.1	45.8	52.7	43.1	44.2
B7	38.6	52.7	48.8	35.5	47.1	44.7
A1	47.1	16.1	21.8	14.7	26.3	25.2
A24	23.9	38.9	58.6	40.1	38.3	40.0
B44	43.0	21.2	42.9	39.1	39.0	37.0
B27	28.4	26.1	13.3	13.9	35.3	23.4
B62	12.6	4.8	36.5	25.4	11.1	18.1
B58	10.0	25.1	1.6	9.0	5.9	10.3
<u>b. Combined Supertypes</u>						
A2, A3, B7	83.0	86.1	87.5	88.4	86.3	86.2
A2, A3, B7, A24, B44, A1	99.5	98.1	100.0	99.5	99.4	99.3
A2, A3, B7, A24, B44, A1, B27, B62, B58	99.9	99.6	100.0	99.8	99.9	99.8

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Table XXII JICY ANALOGS

AA	Sequence	Fixed Nomen.	A1 Motif	A2 Super Motif	A3 Super Motif	A24 Motif	B7 Super Motif	1° Anchor Fixer
9	RVXKMAly		N	N	Y	N	N	
9	AVXTRGVAK		N	N	Y	N	N	
8	EVFXVQPEK		N	N	Y	N	N	
9	HLFXHSKK		N	N	Y	N	N	
9	LPGXFSIF		N	N	N	Y	Y	
9	LFXHSKKK		N	N	Y	N	N	
10	VLAALAYXL		N	Y	N	N	N	No
10	HLFXHSKK		N	N	Y	N	N	
10	AAXNWTRGER		N	N	Y	N	N	
10	YLLPRRGPRV	L2.LV10	N	Y	N	N	N	1
9	FGCSFSIF		N	N	N	N	Y	
9	LPVCSFSIF		N	N	N	N	Y	
9	LPGCSFSYF		N	N	N	N	Y	
9	LPGCMFSIF		N	N	N	N	Y	
9	LPFCFSIF		N	N	N	N	Y	
9	LPGCSFSF		N	N	N	N	Y	
9	LPGCSFSII		N	N	N	N	Y	
9	PPVHGCPI		N	N	N	N	Y	
10	KPTLHGPTI		N	N	N	N	Y	
10	APTLWARMII		N	N	N	N	Y	
9	SPRGSRPSI		N	N	N	N	Y	
9	LPRRGPRLGI		N	N	N	N	Y	
9	SPGQVRFI		N	N	N	N	Y	
9	LPGCSFSII		N	N	N	N	Y	
9	DPRRRSIN		N	N	N	N	Y	
10	SPGALVVGVI		N	N	N	N	Y	
10	TPLLYRLGAI		N	N	N	N	Y	
9	TISGLWQV		N	N	N	N	Y	
9	SISGLWQV		N	Y	N	N	N	No
9	SLMAFTASV		N	Y	N	N	N	No
9	GLRDCIMLV		N	Y	N	N	N	No
10	KLVALGVNAV		N	Y	N	N	N	No
10	YLLPSRGPKL		N	Y	N	N	N	No
10	KLGLGLNAV		N	Y	N	N	N	No
10	YVLRGPRRL		N	Y	N	N	N	No
10	VFFNLGGWV	LV2.L10	N	Y	N	N	N	Rev
10	KLVSGLGVNAV		N	N	N	N	N	No
9	CINGYCWITA	I2.VA9	N	Y	N	N	N	Rev
9	CANGVCWTV	IA2.V9	N	Y	N	N	N	Rev

IICV ANALOGS

AA	Sequence	Fixed Nonvan.	A1 Mottl	A2 Super Mottl	A3 Super Mottl	A24 Mottl	B7 Super Mottl	1° Anchor Fixer
9	CVNGVCHAV 40		N	Y	N	N	N	

Table XXIII. Immunogenicity of identified supermotif-bearing peptides

Supernotif	Peptide	Sequence	Protein	Position	Immunogenicity					Transgenic mice ^b		
					Human ^a							
					Barnaba; patients	Barnaba; contacts	Chisari	Pape	overall	Frequency	Response	
A2	1073.05	LLFNILGGWV	NS4	1812	1/6	7/17	2/21	0/6	10/50	6/6	6.4 (1.7)	
	1090.18	FLLADARV	NS1/E2	728	2/6	7/17	1/21	0/6	10/50	5/6	9.5 (3.0)	
	1013.02	YLVAYQATV	NS4	1590	1/6	4/17	1/21	0/6	6/50	5/6	8.5 (3.7)	
	1090.22	RLIVFPDLGV	NS5	2578	2/6	5/17	0/21	0/6	7/50	0/6	-	
	1013.1002	DLMGYIPLV	Core	132	2/6	7/17	1/21	1/6	11/50	5/6	8.8 (2.6)	
	24.0073	WMNRLIAFA	NS4	1920	1/6	3/17	2/21	1/6	7/50	0/6	-	
	24.0075	VLVGGVLA	NS4	1666	1/6	6/17	3/21	1/6	11/50	0/6	-	
	1174.08	HMWNFISGI	NS4	1769	3/6	3/17	2/21	0/6	8/50	6/6	6.4 (1.7)	
	1073.06	ILAGYGAGV	NS4	1851	2/6	3/17	0/21	0/6	5/50	3/6	54.7 (3.3)	
	1073.07	YLLPRRGPR	CORE	35	2/6	5/17	7/21	1/6	17/50	4/6	59.1 (7.2)	
	24.0071	LLFLLADA	NS1/E2	726	2/6	9/17	0/21	0/6	11/50	0/6	-	
	1.0119	YLVTRHADV	NS3	1131	6/6	10/17	0/21	1/6	17/50	0/6	-	
	A3	1.0952	KTSESRQPR	CORE	51	2/16	1/4	3/12	0/6	6/38	3/6	23.4 (1.3)
		1073.11	RLGVRATRK	CORE	43	4/16	1/4	7/12	1/6	13/38	3/6	42.2 (1.2)
1.0955		QLFTFSPPR	ENV	290	1/16	0/4	6/12	1/6	8/38			
1073.13		RMVVGVEHR	NS1/E2	632	5/16	1/4	4/12	1/6	11/38	2/6	2.8 (1.1)	
1.0123		LIFCHSKK	NS3	1396	6/16	1/4	4/12	2/6	13/38	3/6	4.4 (1.1)	
1073.10		GVAGALVAFK	NS4	1863	3/16	0/4	6/12	2/6	11/38	6/6	56.5 (1.7)	
24.0090		VAGALVAFK	NS4	1864	4/16	1/4	6/12	0/4	11/38	1/6	7.1	
24.0086		TLGFGAYMSK	NS3	1262	6/16		2/12	2/5	10/33			
1143.12		LPGCSFSIF	CORE	169			2	3/10	5			
B7												

Table XXIV. Human and murine MHC-peptide binding assays established using purified MHC molecules and gel filtration chromatography

A. Class I binding assays		Radiolabeled peptide				Notes
Species	Antigen	Allele	Cell line	Source	Sequence	
Human	A1	A*0101	Steinlin	Hu. J chain 102-110	YTAVVPLVY	no NEN in PI cocktail
	A2	A*0201	JY	HBVc 18-27 F6->Y	FLPSDYFSPV	"
	A2	A*0202	P815 (transfected)	HBVc 18-27 F6->Y	FLPSDYFSPV	"
	A2	A*0203	FUN	HBVc 18-27 F6->Y	FLPSDYFSPV	"
	A2	A*0206	CLA	HBVc 18-27 F6->Y	FLPSDYFSPV	"
	A2	A*0207	721.221 (transfected)	HBVc 18-27 F6->Y	FLPSDYFSPV	"
	A3		GM3107	non-natural (A3CON1)	KVFPYALINK	"
	A11		BVR	non-natural (A3CON1)	KVFPYALINK	"
	A24	A*2402	KAS116	non-natural (A24CON1)	AYIDNYNKF	"
	A31	A*3101	SPACH	non-natural (A3CON1)	KVFPYALINK	"
	A33	A*3301	LWAGS	non-natural (A3CON1)	KVFPYALINK	"
	A28/68	A*6801	C1R	HBVc 141-151 T7->Y	STLPETYVVR	"
	A28/68	A*6802	AMAI	HBV pol 646-654 C4->A	FTQAGYPAL	"
	B7	B*0702	GM3107	A2 sigal seq. 5-13 (L7->Y)	APRTLVL	"
	B8	B*0801	Steinlin	IIVgp 586-593 Y1->F, Q5->	FLKDYQLL	"
	B27	B*2705	LG2	R 60s	FRYNGLIHR	"
	B35	B*3501	C1R, BVR	non-natural (B35CON2)	FPFKYAAAF	"
	B35	B*3502	TISI	non-natural (B35CON2)	FPFKYAAAF	"
	B35	B*3503	EHM	non-natural (B35CON2)	FPFKYAAAF	"
	B44	B*4403	PITOUT	EF-1 G6->Y	AEMGKYSFY	"
	B51		KAS116	non-natural (B35CON2)	FPFKYAAAF	"
	B53	B*5301	AMAI	non-natural (B35CON2)	FPFKYAAAF	"
	B54	B*5401	KT3	non-natural (B35CON2)	FPFKYAAAF	"
	Cw4	Cw*0401	C1R	non-natural (C4CON1)	QYDDAVYKL	"
	Cw6	Cw*0602	721.221 transfected	non-natural (C6CON1)	YRHDGGNVL	"
	Cw7	Cw*0702	721.221 transfected	non-natural (C6CON1)	YRHDGGNVL	"
Mouse	D ^b		EL4	Adenovirus E1A P7->Y	SGPSNTYPEI	"
	K ^b		EL4	VSV NP 52-59	RGYVFOGL	"
	D ^d		P815	HIV-IIIIB ENV G4->Y	RGPYRAFVTI	"
	K ^d		P815	non-natural (KdCON1)	KFNPMKTYI	"
	L ^d		P815	HBVs 28-39	IPQSLDSYWTSI	"

Table XXIV. Human and murine MHC-peptide binding assays established using purified MHC molecules and gel filtration chromatography

Species	Antigen	Allele	Cell line	Radiolabeled peptide		Notes
				Source	Sequence	
Human	DR1	DRB1*0101	LG2	HA Y307-319	YPKYVKQNTLKLAT	optimal assay pH is 4.5
	DR2	DRB1*1501	L466.1	MBP 88-102Y	VVHFFKNIVTPRTPPY	
	DR2	DRB1*1601	L242.3	non-natural (760.16)	YAAFAAAKTAATAFA	
	DR3	DRB1*0301	MAT	MT 65kD Y3-13	YKTIADFEEARR	
	DR4w4	DRB1*0401	Preiss	non-natural (717.01)	YARFQSQTTLKQKT	
	DR4w10	DRB1*0402	YAR	non-natural (717.10)	YARFQRQTTLKAAA	
	DR4w14	DRB1*0404	BIN 40	non-natural (717.01)	YARFQSQTTLKQKT	
	DR4w15	DRB1*0405	KT3	non-natural (717.01)	YARFQSQTTLKQKT	
	DR7	DRB1*0701	Pitout	Tet. tox. 830-843	QYIKANSKFIGITE	
	DR8	DRB1*0802	OLL	Tet. tox. 830-843	QYIKANSKFIGITE	
	DR8	DRB1*0803	LUY	Tet. tox. 830-843	QYIKANSKFIGITE	
	DR9	DRB1*0901	HID	Tet. tox. 830-843	QYIKANSKFIGITE	
	DR11	DRB1*1101	Sweig	unknown eluted peptide	EALIHQLKINPYVLS	
	DR12	DRB1*1201	Herluf	Tet. tox. 830-843 S->A	QYIKANAKFIGITE	
	DR13	DRB1*1302	H0301	Tet. tox. 830-843	QYIKANAKFIGITE	
	DR51	DRB5*0101	GM3107 or L416.3	Tet. tox. 830-843	QYIKANAKFIGITE	
	DR51	DRB5*0201	L255.1	HA 307-319	PKYVKQNTLKLAT	
	DR52	DRB3*0101	MAT	Tet. tox. 1272-1284	NGQIGNDPNRDIL	
	DR53	DRB4*0101	L257.6	non-natural (717.01)	YARFQSQTTLKQKT	
	DQ3.1	DQA1*0301/DQB1*0301	PF	non-natural (ROIV)	YAHAAHAAHAAHAAHAA	
Mouse	IA ^b		DB27.4	non-natural (ROIV)	YAHAAHAAHAAHAAHAA	optimal assay pH is 5.5
	IA ^d		A20	non-natural (ROIV)	YAHAAHAAHAAHAAHAA	optimal assay pH is 5.0
	IA ^k		CH-12	HEL 46-61	YNTDGSSTDYGLQNSR	
	IA ^s		LS102.9	non-natural (ROIV)	YAHAAHAAHAAHAAHAA	optimal assay pH is 5.0
	IA ^u		91.7	non-natural (ROIV)	YAHAAHAAHAAHAAHAA	
	IE ^d		A20	Lambda repressor 12-26	YLEDARRKKAIYEKKK	optimal assay pH is 5.0
	IE ^k		CH-12	Lambda repressor 12-26	YLEDARRKKAIYEKKK	optimal assay pH is 5.0

Table XXV. Monoclonal antibodies used in MHC purification.

Monoclonal antibody	Specificity
W6/32	HLA-class I
B123.2	HLA-B and C
IVD12	HLA-DQ
LB3.1	HLA-DR
M1/42	H-2 class I
28-14-8S	H-2 D ^b and L ^d
34-5-8S	H-2 D ^d
B8-24-3	H-2 K ^b
SF1-1.1.1	H-2 K ^d
Y-3	H-2 K ^b
10.3.6	H-2 IA ^k
14.4.4	H-2 IE ^d , IE ^k
MKD6	H-2 IA ^d
Y3JP	H-2 IA ^b , IA ^s , IA ^u

Table XXVI: HCV-derived conserved high algorithm A*0201-binding peptides

Peptide	Molecule	1st Position	Sequence	Consv.	A2-supertype binding capacity (IC50 nM)						A2 XRN
					A*0201	A*0202	A*0203	A*0206	A*6802		
1073.05	NS4	1812	LLFNILGGWV	85	4.2	113	3.2	19	33	5	
1090.18	NS1/E2	728	FLLADARV	92	18	90	149	247	111	5	
1013.02	NS4	1590	YLVAYQATV	85	20	39	16	82	33	5	
1090.22	NS5	2611	RLIVFPDLGV	79	56	391	10	370	8000	4	
1013.1002	CORE	132	DLMGYIPLV	79	80	4778	204	481	12	4	
24.0073	NS4	1920	WMNRLIAFA	100	122	130	3.3	1609	400	4	
24.0075	NS4	1666	VLVGGVLAA	85	185	331	32	308	3077	4	
1174.08	NS4	1769	HMWNFISGI	92	15	10750	77	132	7547	3	
1073.06	NS4	1851	ILAGYGAGV	79	116	143	5.0	755	889	3	
1073.07	CORE	35	YLLPRRGPR	92	125	6143	455	416	10256	3	
24.0071	NS1/E2	726	LLFLLADA	100	217	287	455	3364	3077	3	
1.0119	LORF	1131	YLVTRHADV	85	455	2048	3.6	71	3077	3	
24.0065	NS4	1891	ILSPGALVV	92	238	10750	27	1028	3077	2	
1013.12	NS1/E2	686	ALSTGLIHL	85	313	7167	45	18500	10256	2	
939.14	NS1/E2	696	HLHQNIQVDV	85	500	3071	19	1370	10811	2	
1090.21	NS5	2918	RLHGLSAFSL	79	179	782	625	18500	12500	1	

Table XXVII: HCV-derived conserved high algorithm A*03 and/or A*11 binding peptides

Peptide	Molecule	1st Position	Sequence	Consv.	A3-supertype binding capacity (IC50 nM)						
					A*03	A*11	A*3101	A*3301	A*6801	A3 XRN	
1.0952	CORE	51	KTSESRQPR	92	69	94	67	1813	145	4	
1073.11	CORE	43	RLGVRATRK	79	12	207	429	-	-	3	
1.0955	ENV1	290	QLFTFSRR	79	15	182	621	3766	3	3	
1073.13	NS1/E2	632	RMVVGVEHR	100	15	300	95	9667	1778	3	
1.0123	NS3	1396	LI FCHSKKK	100	20	32	2535	24167	333	3	
1073.10	NS4	1863	GVAGALVAFK	85	28	4	3273	26364	118	3	
24.0090	NS4	1864	VAGALVAFK	85	46	7	3750	11600	258	3	
24.0086	NS3	1262	LGFGAYMSK	85	136	21	2950	22308	222	3	
1174.16	NS1/E2	557	WMNSTGFTK	79	208	74	12857	690	1429	2	
1073.14	NS3	1261	TLGFGAYMSK	85	136	98	-	22308	8889	2	
1090.23	LORF	1183	AVCTRGVAK	79	423	240	16364	-	-	2	
1090.24	NS5	2596	EVFCVQPEK	85	13750	222	-	-	18	2	
24.0103	NS1/E2	647	AACNWTGRG	85	36667	429	400	5273	4444	2	
1073.16	NS3	1232	HLHAPTSGSK	85	19	2500	-	-	2857	1	
1073.12	NS3	1395	HLIFCHSKKK	100	423	-	20000	-	-	1	
1090.26	NS3	1395	HLIFCHSKKK	100	440	10000	-	-	8000	1	

* A dash indicates IC50nM >30,000

Table XXVIII: HCV derived conserved B*0702 binding peptides

A. High conservancy 9- and 10-mer peptides.

Peptide	Molecule	1st Position	Sequence	Consv.	B7-supertype binding capacity (IC50 nM)						
					B*0702	B*3501	B*51	B*5301	B*5401	B7 XRN	
1145.12	Core	169	LPGCSFSIF	92	28	90	100	114	6667	4	
15.0048	E2	681	LPALSTGLI	85	157	-	2.8	1500	20000	2	
15.0234	NS3	1620	KPTLHGPTPL	79	3.9	-	27500	-	-	1	
15.0247	NS5	2835	APTLWARMIL	79	6.3	-	5500	-	-	1	
15.0042	CORE	99	SPRGSRPSW	79	14	-	11000	-	-	1	
15.0039	Core	57	QPRGRRQPI	92	24	-	-	-	-	1	
15.0218	Core	37	LPRRGPRLG	92	29	-	6111	-	4000	1	
15.0060	NS5	2615	SPGQVEFL	79	46	-	27500	-	-	1	
15.0043	Core	111	DPRRRSRNL	85	324	-	-	-	-	1	
15.0063	NS5	2835	APTLWARM	79	344	-	4583	-	-	1	
1292.17	NS5	2317	PPVVHGCPL	79	393	-	-	-	-	1	
15.0239	NS4	1893	SPGALVVG	79	423	-	3438	-	-	1	
15.0235	NS3	1621	TPLLRLGAV	92	458	-	6875	-	909	1	

Table XXVIII: HCV derived conserved B*0702 binding peptides

B. Additional HCV derived B7 supermotif peptides.

Peptide	Molecule	1st Position	Sequence	Consv.	B7-supertype binding capacity (IC50 nM)					
					B*0702	B*3501	B*51	B*5301	B*5401	B7 XRN
29.0035	NS3	1378	IPFYGKAI	92	458	-	46	-	50	3
29.0040	Core	37	LPRRGPR	92	0.85	-	306	-	5000	2
29.0036	Core	137	IPLVGAPL	79	13	2250	79	-	2857	2
16.0187	NS1/E2	680	LPCSFITLPA	64	423	24000	9167	-	15	2
29.0039	Core	169	LPGCSFSI	92	500	200	932	620	6250	2
15.0219	Core	142	APLGAARAL	71	9.5	-	-	-	12500	1
29.0031	NS5	2869	APTLWARM	-	13	-	4583	-	4348	1
15.0231	NS3	1512	RPSGMFDSSV	71	153	-	-	-	-	1
29.0085	NS5	2474	LPINALNSL	57	220	18000	1170	-	11111	1
29.0037	NS5	2608	KPARLIVF	85	367	-	3235	-	16667	1
15.0237	NS4	1789	NPAIASLMAF	71	393	9000	5000	-	-	1
29.0118	NS5	2869	APTLWARMILM	79	423	-	-	-	3030	1
29.0042	NS4	1720	LPYIEQGM	85	423	-	1375	-	7692	1

C. Engineered analogs of B7 supermotif peptides.

Peptide	Molecule	1st Position	Sequence	Consv.	B7-supertype binding capacity (IC50 nM)					
					B*0702	B*3501	B*51	B*5301	B*5401	B7 XRN
1145.12	Core	169	LPGCSFSIF	92	28	90	100	114	6667	4
1292.24	Core	169	LPGCSFSII		37	4364	5.3	262	1056	3
1145.13	Core	169	FPGCSFSIF		19	1.6	132	3.2	6.7	5

* A dash indicates IC50 nM >30,000.

Table XXIX: HCV-derived A1- and A24-motif containing peptides**A. A1-motif peptides**

Peptide	Molecule	Position	Sequence	Conserv.	HLA-A*0101 binding (IC50 nM)
13.0019	NS5	2922	LSAFSLHSY	79	31
1.0509	NS5	2921	GLSAFSLHSY	79	61
1069.62	NS3	1128	CTCGSSDLV	79	68
24.0093	NS5	2129	EVDGVRLLHY	100	167
13.0016	NS3	1241	KSTKVPAAY	85	1923
1.0125	NS3	1525	CYDAGCAWY	79	4032
24.0008	E1	206	DCSNSSIVY	85	16667
24.0094	NS5	2720	TNSKGQNCGY	100	-
24.0096	NS3	1240	GKSTKVPAAY	85	-
24.0100	NS3	1292	TGAPITYSTY	85	-
	NS3	1263	VAATLGFGAY	100	-
	NS5	2639	VMGSSYGFQY	79	-
	NS5	2640	MGSSYGFQY	79	-

A dash indicates IC50 nM >25000

B. A24 -motif peptides

Peptide	Molecule	Position	Sequence	Conserv.	HLA-A*2402 binding (IC50 nM)
24.0092	NS4	1765	FWAKHMWNF	85	1.7
13.0075	NS4	1778	QYLAGLSTL	100	250
1073.18	NS1/E2	636	MYVGGVEHRL	92	444
13.0074	NS3	1297	TYSTYGKFL	85	522
13.0134	NS5	2647	QYSPGQRFV	79	667
24.0091	NS4	1772	NFISGIQYL	100	706
13.0131	Core	135	GYIPLVGAPL	79	2105
24.0108	Core	173	SFSIFLLALL	100	2927
13.0132	NS3	1248	AYAAQGYKVL	79	13333
13.0133	NS4	1859	GYGAGVAGAL	85	-
1174.08	NS4	1769	HMWNFISGI	93	-
	E1	317	RMAWDMMMNW	85	-
	NS1/E2	635	RMYVGGVEHRL	93	-
	NS3	1422	YYRGLDVSVI	100	-
	NS3	1468	DFSLDPTFTI	100	-
	NS3	1608	SWDQMWKCL	79	-
	NS3	1664	TWVLVGGVL	85	-
	NS4	1732	QFKQKALGL	85	-
	NS4	1732	QFKQKALGLL	85	-
	NS4	1765	FWAKHMWNFI	85	-
	NS4	1919	QWMNRLIAF	100	-
	NS5	2241	LWRQEMGGNI	85	-
	NS5	2669	GFSYDTRCF	79	-
	NS5	2875	RMILMTHFF	85	-

A dash indicates IC50 nM >25000

Table XXX: Immunogenicity of A2-supertype cross-reactive binders

Peptide	Sequence	Protein	Position	Immunogenicity						
				Human ^a					Transgenic mice ^b	
				Barnaba; Barnaba;	patients	contacts	Chisari	Pape	overall	Frequency
1073.05	LLFNILGGWV	NS4	1812	1/6	7/17	2/21	0/6	10/50	6/6	6.4 (1.7)
1090.18	FLLADARV	NS1/E2	728	2/6	7/17	1/21	0/6	10/50	5/6	9.5 (3.0)
1013.02	YLVAYQATV	NS4	1590	1/6	4/17	1/21	0/6	6/50	5/6	8.5 (3.7)
1090.22	RLVFPDLGV	NS5	2578	2/6	5/17	0/21	0/6	7/50	0/6	-
1013.1002	DLMGYIPLV	Core	132	2/6	7/17	1/21	1/6	11/50	5/6	8.8 (2.6)
24.0073	WMNRLIAFA	NS4	1920	1/6	3/17	2/21	1/6	7/50	0/6	-
24.0075	VLVGGVLAA	NS4	1666	1/6	6/17	3/21	1/6	11/50	0/6	-
1174.08	HMWNFISGI	NS4	1769	3/6	3/17	2/21	0/6	8/50	6/6	6.4 (1.7)
1073.06	ILAGYGAGV	NS4	1851	2/6	3/17	0/21	0/6	5/50	3/6	54.7 (3.3)
1073.07	YLLPRRGPRRL	CORE	35	2/6	5/17	7/21	1/6	17/50	4/6	59.1 (7.2)
24.0071	LLFLLADA	NS1/E2	726	2/6	9/17	0/21	0/6	11/50	0/6	-
1.0119	YLVTRHADV	NS3	1131	6/6	10/17	0/21	1/6	17/50	0/6	-

a. Data shown represents the number of positive responses over the total number of patients or contacts examined.

b. Frequency represents the number of positive responses over the total number of mice examined. Response indicates the average magnitude (standard deviation) of the response in positive animals, measured in lytic units.

Table XXXI: Immunogenicity of A3-supertype cross-reactive binders

Peptide	Sequence	Protein	Position	Immunogenicity					
				Human ^a			Transgenic mice ^b		
				Barnaba patients	Barnaba contacts	Chisari	Pape	overall	Frequency Response
1.0952	KTSESRQR	CORE	51	2/16	1/4	3/12	0/6	6/38	3/6 23.4 (1.3)
1073.11	RLGVRATRK	CORE	43	4/16	1/4	7/12	1/6	13/38	3/6 42.2 (1.2)
1.0955	QLFTFSRR	ENV	290	1/16	0/4	6/12	1/6	8/38	
1073.13	RMVVGVEHR	NS1/E2	632	5/16	1/4	4/12	1/6	11/38	2/6 2.8 (1.1)
1.0123	LIFCHSKKK	NS3	1396	6/16	1/4	4/12	2/6	13/38	3/6 4.4 (1.1)
1073.10	GVAGALVAFK	NS4	1863	3/16	0/4	6/12	2/6	11/38	6/6 56.5 (1.7)
24.0090	VAGALVAFK	NS4	1864	4/16	1/4	6/12	0/4	11/38	1/6 7.1
24.0086	TLGFGAYMSK	NS3	1262	6/16		2/12	2/5	10/33	

a. Data shown represents the number of positive responses over the total number of patients or contacts examined.

b. Frequency represents the number of positive responses over the total number of mice examined. Response indicates the average magnitude (standard deviation) of the response in positive animals, measured in lytic units.

Table XXXII. Candidate HCV-derived HTL epitopes

Selection criteria	Peptide	Sequence	Source	Conservancy	
				Total	Core
A. DR-supermotif conserved 15mers	1283.01	GQIVGGVYLLPRRGPR	HCV Core 28	93	93
	1283.02	VYLLPRRGPRLGVR	HCV Core 34	93	93
	1283.03	GWLLSPRGSRPSWGPT	HCV Core 95	79	79
	1283.04	LGKVIDTLTCGFADL	HCV Core 119	79	86
	1283.05	IDTLTCGFADLMGYI	HCV Core 123	86	86
	1283.06	ADLMGYIPLVGAPLG	HCV Core 131	79	79
	1283.07	GVRVLEDGVNYATGN	HCV Core 154	86	86
	1283.08	GNYATGNLPGCSFS	HCV Core 161	79	86
	1283.09	GCSFSIFLLALLSCL	HCV Core 171	86	100
	1283.10	GHRMAWDMMMNWSPT	HCV E1 315	86	86
	1283.11	CGPVYCFTPSPVVVG	HCV NS1/E2 506	93	93
	1283.12	VYCFTSPVVGTTD	HCV NS1/E2 509	93	93
	1283.13	GNWFGCTWMNSTGFT	HCV NS1/E2 550	79	86
	1283.14	FTTLPALSTGLHLH	HCV NS1/E2 684	79	86
	1283.17	DLYLVTRHADVIPVR	HCV NS3 1134	79	79
	1283.18	RAAVCTRGVAKAVDF	HCV NS3 1186	79	79
	1283.20	AQGYKVLVLNPSVAA	HCV NS3 1251	79	100
	1283.21	GYKVLVLNPSVAATL	HCV NS3 1253	100	100
	1283.22	VLVLNPSVAATLGFG	HCV NS3 1256	100	100
	1283.23	GTVLDQAETAGARLV	HCV NS3 1335	86	86
	1283.24	GARLVVLAATATPPGS	HCV NS3 1345	79	86
	1283.25	GRHLIFCHSKKCCDE	HCV NS3 1393	100	100
	1283.27	DSVIDCNTCVTQTVD	HCV NS3 1454	86	86
	1283.28	TVDFSLDPTFTIETT	HCV NS3 1466	79	100
	1283.30	FTGLTHIDAHFLSQT	HCV NS3 1567	93	93
	1283.31	YLVAAYQATVCARAQA	HCV NS3 1591	79	93
	1283.32	KPTLHGPTPLLYRLG	HCV NS4 1620	79	79
	1283.33	LEVVTSTWVLVGGVL	HCV NS4 1658	86	86
	1283.34	TWVLVGGVLAALAAAY	HCV NS4 1664	86	86
	1283.35	AEQFKQKALGLLQTA	HCV NS4 1730	86	86
	1283.40	PAILSPGALVVGVCVA	HCV NS4 1889	79	93
	1283.41	GALVVGVCVAAILRR	HCV NS4 1895	79	79
	1283.42	CAAILRRHVGPGEA	HCV NS4 1903	79	79
	1283.43	AVQWMNRLIAFASRG	HCV NS4 1917	100	100
	1283.44	MNRLIAFASRGNHVS	HCV NS4 1921	86	100
	1283.48	ANLLWRQEMGGNITR	HCV NS5 2238	86	86
	1283.49	RQEMGGNITRVESEN	HCV NS5 2243	86	86
	1283.52	ARLIVFPDLGVRVCE	HCV NS5 2610	79	79
	1283.53	FDDLGVRCCEKMALY	HCV NS5 2615	79	100
	1283.54	GVRVCEKMALYDVVS	HCV NS5 2619	79	100
	1283.56	QPEYDLELITSCSSN	HCV NS5 2808	79	93
	1283.57	LELITSCSSNVSVAH	HCV NS5 2813	79	100
	1283.58	PTLWARMILMTHFFS	HCV NS5 2870	79	86
	1283.59	LHGLSAFSLHSYSPG	HCV NS5 2919	79	79
	1283.60	AFSLHSYSPGEINRV	HCV NS5 2924	79	79

Table XXXII. Candidate HCV-derived HTL epitopes

Selection criteria	Peptide	Sequence	Source	Conservancy	
				Total	Core
B. High algorithm conserved core	1283.15	VVLLFLLADARVCS	HCV NS1/E2 724	29	100
	1283.16	SKGWRLLAPITAYAQ	HCV NS3 1025	29	79
	1283.19	PQTFQVAHLHAPTGS	HCV NS3 1225	43	85
	1283.26	DVVVVATDALMTGYT	HCV NS3 1436	43	79
	1283.29	WESVFTGLTHIDAHF	HCV NS3 1563	43	92
	1283.45	LTSMLTDP SHITAET	HCV NS5 2176	57	100
	1283.46	ASQLSAPSLKATCTT	HCV NS5 2208	50	79
	1283.47	DADLIEANLLWRQEM	HCV NS5 2232	50	85
	1283.50	SYTWTGALITPCAAE	HCV NS5 2456	64	79
	1283.51	TTIMAKNEVFCVQPE	HCV NS5 2589	64	85
	1283.55	GSSYGFQYSPGQ RVE	HCV NS5 2641	71	79
	1283.61	ASCLRKLGVPLRVW	HCV NS5 2939	50	85
C. Collaborator	F098.03	AAYAAQGYKVLV LNPSVAAT	HCV NS3 1242-1261	71	100
	F098.04	GYKVLV LNPSVAATLGFGAY	HCV NS3 1248-1267	100	
	F098.05	GYKVLV LNPSVAAT	HCV NS3 1248-1261	100	
	F134.01	RRPQDV KFPGGGQIVGGVY	HCV Core 17-35	86	
	F134.02	DVKFPGGGQIVGGVYLLPRR	HCV Core 21-40	86	
	F134.03	GYKVLV LNPSVAATLGFGAY	HCV NS3 1253-1272	100	
	F134.04	TLHGPTPLL YRLGAVQNEIT	HCV NS4 1622-1641		79
	F134.05	NFISGIQYLAGLSTLPGNPA	HCV NS4 1772-1791	100	
	F134.06	LLFNILGGWVAAQLAAPGAA	HCV NS4 1812-1831		86
	F134.07	GPGE GAVQWMNRLIAFASRG	HCV NS4 1912-1931	86	100
	F134.08	GEGAVQWMNRLIAFASRG NHV	HCV NS4 1914-1934	100	
	Pape 21	AIPLEV KGGRH LIFCHSKR	HCV NS3 1379-1398	21	100
	Pape 22	GRHLIFCHSKRK CDE LATKL	HCV NS3 1388-1407		100
	Pape 29	SVIDCNTCVTQT VDFSLDPT	HCV NS3 1450-1469	86	
D. DR3 motif	35.0102	GVRVLEDGVNYATGN	HCV 154	86	86
	35.0103	SAMYVGDL CGSVFLV	HCV 273	57	86
	35.0104	GHRMAWDM MNWSP T	HCV 315	86	86
	35.0105	SDLYLVTRHADVIPV	HCV 1133	79	86
	35.0106	VVVVATDALMTGYTG	HCV 1437	42	86
	35.0107	TVDFSLDPTFTIETT	HCV 1466	79	100
	35.0108	DSSVLCECYDAGCAW	HCV 1518	71	93
	35.0109	GLPVCQDHLEFWESV	HCV 1552	42	86
	35.0110	GMQLAEQFKQKALGL	HCV 1726	57	86
	35.0111	PTHYVPESDAAARVT	HCV 1936	86	86
	35.0112	GSQLPCEPEPDVAVL	HCV 2162	64	86
	35.0113	LTSMLTDP SHITAET	HCV 2176	57	100
	35.0114	MPPLEGE PGDPDLS D	HCV 2401	79	100
	35.0115	QPEYDLELITSCSSN	HCV 2808	79	93
	1283.25	GRHLIFCHSKKK CDE	HCV NS3 1393-1407		

Table XXXIII. HLA-DR screening panels

Screening Panel	Antigen	Alleles	Representative Assay		Phenotypic Frequencies					
			Allele	Alias	Cauc.	Blk.	Jpn.	Chm.	Hisp.	Avg.
Primary	DR1	DRB1*0101-03	DRB1*0101	(DR1)	18.5	8.4	10.7	4.5	10.1	10.4
	DR4	DRB1*0401-12	DRB1*0401	(DR4w4)	23.6	6.1	40.4	21.9	29.8	24.4
	DR7	DRB1*0701-02	DRB1*0701	(DR7)	26.2	11.1	1.0	15.0	16.6	14.0
	Panel total				59.6	24.5	49.3	38.7	51.1	44.6
Secondary	DR2	DRB1*1501-03	DRB1*1501	(DR2w2 B1)	19.9	14.8	30.9	22.0	15.0	20.5
	DR2	DRB5*0101	DRB5*0101	(DR2w2 B2)	-	-	-	-	-	-
	DR9	DRB1*09011,09012	DRB1*0901	(DR9)	3.6	4.7	24.5	19.9	6.7	11.9
	DR13	DRB1*1301-06	DRB1*1302	(DR6w19)	21.7	16.5	14.6	12.2	10.5	15.1
	Panel total				42.0	33.9	61.0	48.9	30.5	43.2
Tertiary	DR4	DRB1*0405	DRB1*0405	(DR4w15)	-	-	-	-	-	-
	DR8	DRB1*0801-5	DRB1*0802	(DR8w2)	5.5	10.9	25.0	10.7	23.3	15.1
	DR11	DRB1*1101-05	DRB1*1101	(DR5w11)	17.0	18.0	4.9	19.4	18.1	15.5
	Panel total				22.0	27.8	29.2	29.0	39.0	29.4
Quarternary	DR3	DRB1*0301-2	DRB1*0301	(DR3w17)	17.7	19.5	0.4	7.3	14.4	11.9
	DR12	DRB1*1201-02	DRB1*1201	(DR5w12)	2.8	5.5	13.1	17.6	5.7	8.9
	Panel total				20.2	24.4	13.5	24.2	19.7	20.4

Table XXXIV. HLA-DR binding capacity of target derived peptides: DR-supermotif and algorithm positive peptides.

Peptide	Sequence	Source	Binding capacity (IC50 nM)										DR alleles	
			DR1	DR2w281	DR2w282	DR4w4	DR4w15	DR3w11	DR6w19	DR7	DR8w2	DR9	IAb	bound
1283.21	AAYAAQGYKVLNPSVAATLGFAY	HCV NS3 1242-1267	4.5	350		5.2	567	143	5.1	89	288	54	175	9
1283.20	GYKVLNPSVAATL	HCV NS3 1253	6.0	650		7.9	224	74	5.9	833	175	375	298	9
F98.03	AQGYKVLNPSVAA	HCV NS3 1251	2.9	48	483	18	1234	103	1.1	96	60	240		9
F98.05	AAYAAQGYKVLNPSVAAT	HCV NS3 1242	1.4	39	3695	7.8	141	75	3.5	126	21	266		9
F98.04	GYKVLNPSVAAT	HCV NS3 1248-1261	3.5	42	3154	9.7	500	240	4.1	23	80	20		8
	GYKVLNPSVAATLGFAY	HCV NS3 1248-1267												
	GEGAVQWMNRLIAFASRGNHVS	HCV NS4 1914-1935												
1283.44	MNRLIAFASRGNHVS	HCV NS4 1921	66	4.8	1538	6329	585	45	7.3	227	102	313	147	8
F134.08	GEGAVQWMNRLIAFASRGNHV	HCV NS4 1914	3.2		182	361	345	345		221	158	6818		6
1283.16	SKGWRLLAPITAYAQ	HCV NS3 1025	0.36	125	23	24	152	4.8		962	54	1190	384	8
1283.55	GSSYGFQYSPQORVE	HCV NS5 2641	11		667	417	745	20000	19	156		68	571	7
1283.61	ASCLRKLGVPLRVW	HCV NS5 2939	5.0	16	217	6250	78	645	2500	862	671	862		7
F134.05	NFISGIQYLAGLSTLPGNPA	HCV NS4 1772	10		606	84		29			70	441		6

Shading indicates IC50 > 1 μM.

A dash (-) indicates IC50 > 20 μM.

Table XXXV. HLA-DR binding capacity of 3 DR3 motifs containing peptides

Peptide	Sequence	Source	DR3 binding (IC50 nM)
35.0106	VVVVATDALMTGYTG	HCV 1437	427
35.0107	TVDFSLDPTFTIET	HCV 1466	235
1283.25	GRHLIFCHSKKKCDE	HCV NS3 1393	ND

Table XXXVIa: HCV-derived CTL epitope candidates

Peptide	Molecule	1st Position	Sequence	Consv.	Selection criteria
1073.05	NS4	1812	LLFNILGGWV	85	A2-supertype
1090.18	NS1/E2	728	FLLLDARV	92	A2-supertype
1013.02	NS4	1590	YLVAYQATV	85	A2-supertype
1090.22	NS5	2611	RLVFPDLGV	79	A2-supertype
1013.1002	CORE	132	DLMGYIPLV	79	A2-supertype
24.0073	NS4	1920	WMNRLJFA	100	A2-supertype
24.0075	NS4	1666	VLVGGVLAA	85	A2-supertype
1174.08	NS4	1769	HMWNFISGI	92	A2-supertype
1073.06	NS4	1851	ILAGYGAGV	79	A2-supertype
1073.07	CORE	35	YLLPRRGPR	92	A2-supertype
24.0071	NS1/E2	726	LLFLLADA	100	A2-supertype
1.0119	LORF	1131	YLVTRHADV	85	A2-supertype
1.0952	CORE	51	KTSESRQPR	92	A3-supertype
1073.11	CORE	43	RLGVRATRK	79	A3-supertype
1.0955	ENV1	290	QLFTFSRR	79	A3-supertype
1073.13	NS1/E2	632	RMVYVGGVEHR	100	A3-supertype
1.0123	NS3	1396	LIFCHSKKK	100	A3-supertype
1073.10	NS4	1863	GVAGALVAFK	85	A3-supertype
24.0090	NS4	1864	VAGALVAFK	85	A3-supertype
24.0086	NS3	1262	TLGFGAYMSK	85	A3-supertype
F104.01	NS5	3003	VGIYLLPNR	79	A31
1145.12	Core	169	LPGCSFSIF	92	B7-supertype
29.0035	NS3	1378	IPFYGKAI	92	B7-supertype
13.0019	NS5	2922	LSAFSLHSY	79	A1
1069.62	NS3	1128	CTCGSSDLY	79	A1
24.0092	NS4	1765	FWAKHMMWNF	85	A24

Table XXXVIb: HCV-derived HTL epitope candidates

Region	Peptide	Motif ¹	Sequence
HCV NS3 1025-1039	1283.16	DR	SKGWRLLAPITAYAQ \
HCV NS3 1242-1267	F98.03	DR	AAVAAQGYKVLVLPNSVAAT \
HCV NS3 1393-1407	1283.25	DR3	GRHLIFCHSKKKCDE \
HCV NS3 1437-1451	35.0106	DR3	VVVVATDALMTGYTG \
HCV NS3 1466-1480	35.0107	DR3	TVDFSLDPTFTIETT \
HCV NS4 1772-1790	F134.05	DR	NFISGIQYLAGLSTLPGNPA \
HCV NS4 1914-1935	F134.08	DR	GEGAVQWMNRLIAFASRGNHV \
HCV NS5 2641-2655	1283.55	DR	GSSYGFQYSPGQERVE \
HCV NS5 2939-2953	1283.61	DR	ASCLRKLGVPPLRVW \

1. Peptides identified on the basis of either the DR P1-P6 supermotif or by use of the DR1-4-7 algorithms are indicated by 'DR'. Peptides identified using the DR3 motif are indicated by 'DR3'.

Table XXXVII. Estimated population coverage by a panel of HCV derived HTL epitopes

Antigen	Alleles	Representative assay	No. of epitopes ²	Population coverage (phenotypic frequency)					
				Cauc.	Blk.	Jpn.	Chn.	Hisp.	Avg.
DR1	DRB1*0101-03	DR1	6	18.5	8.4	10.7	4.5	10.1	10.4
DR2	DRB1*1501-03	DR2w2 B1	3	19.9	14.8	30.9	22.0	15.0	20.5
DR2	DRB5*0101	DR2w2 B2	6	-	-	-	-	-	-
DR3	DRB1*0301-2	DR3	2	17.7	19.5	0.40	7.3	14.4	11.9
DR4	DRB1*0401-12	DR4w4	5	23.6	6.1	40.4	21.9	29.8	24.4
DR4	DRB1*0401-12	DR4w15	3	-	-	-	-	-	-
DR7	DRB1*0701-02	DR7	5	26.2	11.1	1.0	15.0	16.6	14.0
DR8	DRB1*0801-5	DR8w2	5	5.5	10.9	25.0	10.7	23.3	15.1
DR9	DRB1*09011,09012	DR9	3	3.6	4.7	24.5	19.9	6.7	11.9
DR11	DRB1*1101-05	DR5w11	5	17.0	18.0	4.9	19.4	18.1	15.5
DR13	DRB1*1301-06	DR6w19	2	21.7	16.5	14.6	12.2	10.5	15.1
Total ¹				98.5	95.1	97.1	91.3	94.3	95.1

1. Total population coverage has been adjusted to account for the presence of DRX in many ethnic populations. It has been assumed that the range of specificities represented by DRX alleles will mirror those of previously characterized HLA-DR alleles. The proportion of DRX incorporated under each motif is representative of the frequency of the motif in the remainder of the population. Total coverage has not been adjusted to account for unknown gene types.

2. Number of epitopes represents a minimal estimate, considering only the epitopes shown in Table 6. Additional alleles possibly bound by nested epitopes have not been accounted.

TABLE Ia

SUPERMOTIFS	POSITION	POSITION	POSITION
	2 (Primary Anchor)	3 (Primary Anchor)	C Terminus (Primary Anchor)
A1	T , I , <i>L</i> , <i>V</i> , <i>M</i> , <i>S</i>		F , W , Y
A2	V , <i>Q</i> , <i>A</i> , <i>T</i>		I , V , <i>L</i> , <i>M</i> , <i>A</i> , <i>T</i>
A3	V , S , M , A , <i>T</i> , <i>L</i> , <i>I</i>		R , K
A24	Y , F , <i>W</i> , <i>I</i> , <i>V</i> , <i>L</i> , <i>M</i> , <i>T</i>		F , I , <i>Y</i> , <i>W</i> , <i>L</i> , <i>M</i>
B7	P		V , I , L , F , <i>M</i> , <i>W</i> , <i>Y</i> , <i>A</i>
B27	R , H , K		F , Y , L , <i>W</i> , <i>M</i> , <i>I</i> , <i>V</i> , <i>A</i>
B58	A , T , S		F , W , Y , <i>L</i> , <i>I</i> , <i>V</i> , <i>M</i> , <i>A</i>
B62	Q , L , <i>I</i> , <i>V</i> , <i>M</i> , <i>P</i>		F , W , Y , <i>M</i> , <i>I</i> , <i>V</i> , <i>L</i> , <i>A</i>
MOTIFS			
A1	T , S , M		Y
A1		D , E , <i>A</i> , <i>S</i>	Y
A2.1	<i>V</i> , <i>Q</i> , <i>A</i> , <i>T</i> *		V , <i>L</i> , <i>I</i> , <i>M</i> , <i>A</i> , <i>T</i>
A3.2	L , M , V , I , S , A , T , F , <i>C</i> , <i>G</i> , <i>D</i>		K , Y , R , <i>H</i> , <i>F</i> , <i>A</i>
A11	V , T , M , L , I , S , A , G , N , <i>C</i> , <i>D</i> , <i>F</i>		K , R , H , Y
A24	Y , F , W		F , L , I , W

*If 2 is V, or Q, the C-term is not L

Bolded residues are preferred, italicized residues are less preferred: A peptide is considered motif-bearing if it has primary anchors at each primary anchor position for a motif or supermotif as specified in the above table.

WHAT IS CLAIMED IS

1. A composition comprising a prepared hepatitis C virus (HCV) epitope consisting of an amino acid sequence selected from the group consisting of :

FLLLADARV,	YLVAYQATV,	RLIVFPDLGV,
DLMGYIPLV,	WMNRLIAFA,	VLVGGVLAA,
HMWNFISGI,	ILAGYGAGV,	YLLPRRGPR,
LLFLLLADA,	YLVTRHADV,	KTSEERSQPR,
RLGVRATRK,	QLFTFSPRR,	RMVVGGVVEHR,
LIFCHSKKK,	GVAGALVAFK,	VAGALVAFK,
TLGFGAYMSK,	LPGCSFSIF,	LSAFSLHSY,
CTCGSSDLY,	FWAKHMWNF,	SKGWRLAPITAYAQ,
AAAYAAQGYKVLVLNPSVAAT,	GRHLIFCHSKKKCDE,	VVVVATDALMTGYTG,
TVDFSLDPTFTIETT,	NFISGIQYLAGLSTLPGNPA,	
GEGAVQWMNRLIAFASRGNHV, GSSYGFQYSPGQQRVE, ASCLRKLGVPLRVW,		
and LTCGFADLMGY.		

2. The composition of claim 1, further comprising two epitopes selected from the group in claim 1.

3. The composition of claim 2, further comprising three epitopes selected from the group in claim 1.

4. The composition of claim 1, wherein the composition further comprises a CTL epitope selected from the group consisting of LTDPSHITA, LADGGCSGGAY, RMILMTHFF, VMGSSYGF, FWAKHMWNFI, LLFNILGGWV, IPFYGKAI, and VGIYLLPNR.

5. The composition of claim 1, wherein the composition further comprises an HTL epitope.

6. The composition of claim 5, wherein the HTL epitope is a pan DR binding molecule.

7. The composition of claim 1, wherein the epitope is on or within a liposome.
8. The composition of claim 1, wherein the peptide is joined to a lipid.
9. The composition of claim 1, wherein the epitope is bound to an HLA heavy chain, β 2-microglobulin, and streptavidin complex, whereby a tetramer is formed.
10. The composition of claim 1, wherein the epitope is bound to an HLA molecule on an antigen presenting cell.
11. The composition of claim 10, wherein the antigen presenting cell is a dendritic cell.
12. The composition of claim 1, the composition further comprising a pharmaceutical excipient.
13. The composition of claim 1, further wherein the epitope is in a unit dose form.
14. A composition comprising a prepared peptide of less than 250 amino acid residues comprising at least two hepatitis C virus (HCV) peptide epitopes selected from the group consisting of:
- | | | |
|-------------|-------------|-----------------|
| FLLLADARV, | YLVAYQATV, | RLIVFPDLGV, |
| DLMGYIPLV, | WMNRLIAFA, | VLVGGVLAA, |
| HMWNFISGI, | ILAGYGAGV, | YLLPRRGPR, |
| LLFLLADA, | YLVTRHADV, | KTSESRQPR, |
| RLGVRATRK, | QLFTFSPRR, | RMVVGVEHR, |
| LIFCHSKKK, | GVAGALVAFK, | VAGALVAFK, |
| TLGFGAYMSK, | LPGCSFSIF, | LSAFSLHSY, |
| CTCGSSDLY, | FWAKHMWNF, | SKGWRLAPITAYAQ, |

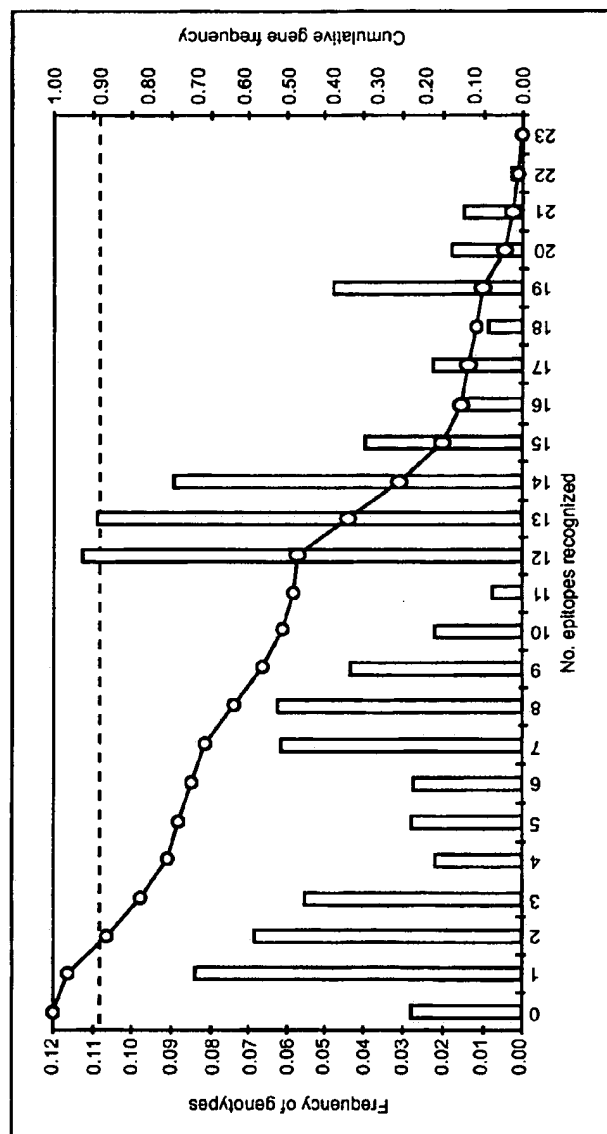
AAAYAAQGYKVLVLNPSVAAT, GRHLIFCHSKKKCDE, VVVVATDALMTGYTG,
TVDFSLDPTFTIETT, NFISGIQYLAGLSTLPGNPA,
GEGAVQWMNRLIAFASRGNHV, GSSYGFQYSPGQRVE, ASCLRKLGPPLRVW,
and LTCGFADLMGY.

15. The composition of claim 14, wherein at least two epitopes are linked via a spacer.
16. The composition of claim 14, further comprising a third epitope.
17. The composition of claim 16, wherein the third epitope is selected from the group consisting of LTDPSHITA, LADGGCSGGAY, RMILMTHFF, VMGSSYGF, FWAKHMWNFI, LLFNILGGWV, IPFYGKAI, and VGIYLLPNR.
18. The composition of claim 16, further comprising a third epitope that is an HTL epitope.
19. The composition of claim 18, wherein the HTL epitope is a panDR binding molecule.
20. The composition of claim 14, wherein the peptide is on or within a liposome.
21. The composition of claim 14, wherein the peptide is joined to a lipid.
22. The composition of claim 14, wherein the peptide further comprises at least three of the epitopes in the group of claim 14.
23. The composition of claim 14, wherein the peptide further comprises at least four of the epitopes in the group of claim 14.
24. The composition of claim 14, wherein the peptide further comprises at least five of the epitopes in the group of claim 14.

25. The composition of claim 14, wherein the peptide further comprises at least six of the epitopes in the group of claim 14.
26. The composition of claim 14, the composition further comprising a pharmaceutical excipient.
27. The composition of claim 14, further wherein the epitope is in a unit dose form.
28. A composition comprising at least six prepared HCV epitopes each consisting of an amino acid sequence selected from the group consisting of:
- | | | |
|------------------------|-----------------------|------------------|
| FLLLADARV, | YLVAYQATV, | RLIVFPDLGV, |
| DLMGYIPLV, | WMNRLIAFA, | VLVGGVLAA, |
| HMWNFISGI, | ILAGYGAGV, | YLLPRRGPR, |
| LLFLLADA, | YLVTRHADV, | KTSESRQPR, |
| RLGVRATRK, | QLFTFSPRR, | RMVVGVEHR, |
| LIFCHSKKK, | GVAGALVAFK, | VAGALVAFK, |
| TLGFGAYMSK, | LPGCSFSIF, | LSAFSLHSY, |
| CTCGSSDLY, | FWAKHMWNF, | SKGWRLAPITAYAQ, |
| AAAYAAQGYKVLVLNPSVAAT, | GRHLIFCHSKKKCDE, | VVVVATDALMTGYTG, |
| TVDFSLDPTFTIETT, | NFISGIQYLAGLSTLPGNPA, | |
| GEGAVQWMNRLIAFASRGNHV, | GSSYGFQYSPGQRVE, | ASCLRKLGPPLRVW, |
- and LTCGFADLMGY.
29. The composition of claim 28, further comprising at least one epitope selected from the group consisting of LTDP SHITA, LADGGCSGGAY, RMILMTHFF, VMGSSYGF, FWAKHMWNFI, LLFNILGGWV, IPFYGKAI, and VGIYLLPNR.

1 / 2

Monte Carlo population coverage analysis for HCV candidate epitopes



Plot of total frequency of genotypes as a function of the number of HCV candidate epitopes bound by HLA-A and B alleles, in an average population. Genotype values were derived by averaging the gene frequencies in Caucasian, North American, Black, Japanese, Chinese, and Hispanic populations. Also shown is the cumulative frequency of genotypes.

Using currently available HLA typing data, a residual fraction (about 15%) of the genes, in an average population, are unspecified. To arrive at 100% accounting of genes, a fraction of the residual has been added for each hit population cluster in proportion to the relative frequency of the cluster within the HLA specified population. One peptide, 24.0086, was not incorporated into the present analysis.

FIG. 1

HVC Minigene

CTL Epitopes

Core 43		NS4 1590	NS3 1128	NS5 2611	Core 169	NS1/E2 632	NS4 1765	NS4 1863	Core 132
Kozak	SigSeq	1073.11	1013.02	1069.62	1090.02	1145.12	1073.13	24.0092	1073.10
A3		A2	A1	A2	B7	A3	A24	A3	A2

NS3 1253	NS4 1921	1437	NS5 2641	1466
1283.21	1283.44	35.0106	1283.55	35.0107
DR	DR	DR3	DR	DR3

HTL Epitopes

FIG. 2

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/19774

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 38/00, 38/04, 38/08, 38/10, 39/29, 39/295

US CL : 514/2, 12, 13, 14, 15, 885; 424/185.1, 189.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/2, 12, 13, 14, 15, 885; 424/185.1, 189.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE, BIOSIS, EMBASE, DERWENT WPI, WEST 2.0, search terms: author names, hcv, peptid?, HLA, htl, ctl.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim N
Y	WENTWORTH et al. Differences and similarities in the A2.1-restricted cytotoxic T cell repertoire in humans and human leukocyte antigen-transgenic mice. Eur. J. Immunol. 1996. Vol 26. pages 97-101, see entire document.	1-29
Y	US 5,736,142 A (SETTE et al.) 07 April 1998, see entire document.	1-29



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or prior date and not in conflict with the application but cited to understate the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document combined with one or more other such documents, such combinations being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*G* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

19 SEPTEMBER 2000

Date of mailing of the international search report

17 OCT 2000

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